A

Dissertation on

"Evaluation of serum zinc status and serum alkaline phosphatase activity in alcoholic liver disease"

Submitted to the

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY

In partial fulfilment of the requirements

For the award of degree of

M.D. (Branch-XIII)

BIOCHEMISTRY

GOVERNMENT STANLEY MEDICAL

COLLEGE & HOSPITAL

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY,

CHENNAI, TAMILNADU

APRIL 2016

CERTIFICATE

This is to certify that the dissertation **"Evaluation of serum zinc status and serum alkaline phosphatase activity in alcoholic liver disease "** presented herein by **DR.T.UMA,** is an original work done in the Department of Biochemistry,Government Stanley Medical college and Hospital,Chennai, in partial fulfillment of regulations of The Tamilnadu Dr.M.G.R Medical university for the award degree of M.D(Biochemistry) Branch XIII,during the academic year 2013-2016.

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DECLARATION

I, **Dr.T.UMA** solemnly declare that this dissertation, titled **Evaluation of serum zinc status and serum alkaline phosphatase activity in alcoholic liver disease** ″ is a bonafide record of work done by me in the Department of Biochemistry,Stanley Medical College and Hospital,Chennai under the guidance of **Dr.R.SHANTHI M.D., DCP** Associate Professor, Department of Biochemistry, Government Stanley Medical College & Hospital,Chennai-600001.

This dissertation is submitted to The Tamilnadu Dr.M.G.R Medical University,Chennai in partial fulfillment of the University regulations for the award of degree of M.D (Biochemistry),Branch XIII,Examination to be held in April 2016.

Place : Chennai

Date : **Dr.T.UMA**

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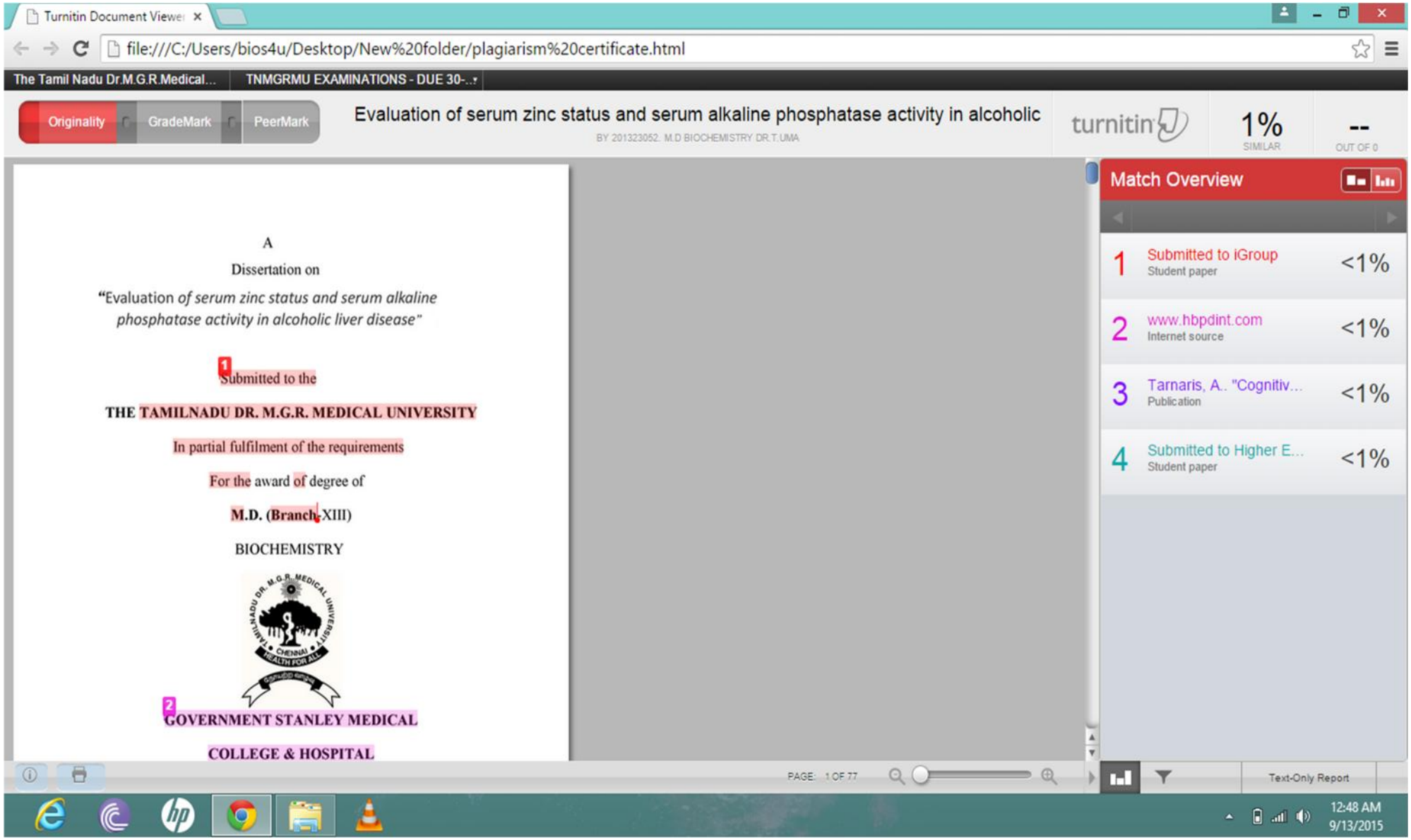
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ABBREVIATIONS

ALD –Alcoholic liver disease

AST – Aspartate Transaminase

ALT – Alanine Transaminase

GGT- Gamma Glutamyl Transferase

CDT- Carbohydrate Deficient Protein

WHO- World Health Organisation

DALYs- Disability Adjusted Life Years

ALDH –Aldehyde Dehydrogenase

ADH – Alcohol Dehydrogenase

PPAR – Peroxisome Proliferator – Activated Receptor

ROS- Reactive Oxygen Species

ACA- Acetaldehyde Adducts

TAG – Triacyl glycerol

NAD- Nicotinamide Adenine Dinucleotide

NADH-Nicotinamide Adenine Dinucleotide (Reduced)

HDL – High Density Lipoproteins

FFA- Free Fatty Acids

TNF – Tumor Necrosis Factor

MDH – Malate Dehydrogenase

IFCC- International Federation of Clinical Chemistry

LDH- Lactate Dehydrogenase

BCG – Bromo Cresol Green

U/L – Units per Litre

INTRODUCTION

Alcohol consumed by all strata of society remains to be a major cause for morbidity and mortality worldwide. Unquestionably ethanol is a hepatotoxic compound that leads to serious form of alcoholic liver disease[1].

Chronic alcoholism results in changes of the intestinal epithelial barrier, increase pro-inflammatory cytokine production, generation of ROS which are the key factors in mediating alcoholic liver disease[2].

Zinc deficiency has been documented with alcoholic liver disease moreover the decrease in serum zinc correlates with the progression of liver disease[3].

It is also a well-known fact that zinc is a co-factor for enzyme alkaline phosphatase. This study aims at assessing the zinc status and alkaline phosphatase in patients with alcoholic liver disease.

AIMS AND OBJECTIVES

The aim is to evaluate zinc and alkaline phosphatase activity in patients with alcoholic liver disease.

The objectives are

- To determine serum zinc.
- To estimate alkaline phosphatase activity.
- To assess the correlation between zinc and alkaline phosphatase activity.
- To analyze the state of zinc in various stages of alcoholic liver disease.
- To estimate the need for evaluating zinc status and supplementing zinc in patients with alcoholic liver disease.

ALCOHOLIC LIVER DISEASE-EPIDEMIOLOGY

Alcohol attributable injuries and negative impacts are now becoming the major concern to the health of the public.

Consuming alcoholic beverages is the potent etiological factor for the development of alcoholic liver diseases(ALD), ranging from fatty liver to hepatocellular carcinoma with varying rates of development in both genders depending on the quality , quantity and duration of the drink.

According to Global Information System on Alcohol and Health (GISAH) data,3.3 million people die annually[4].

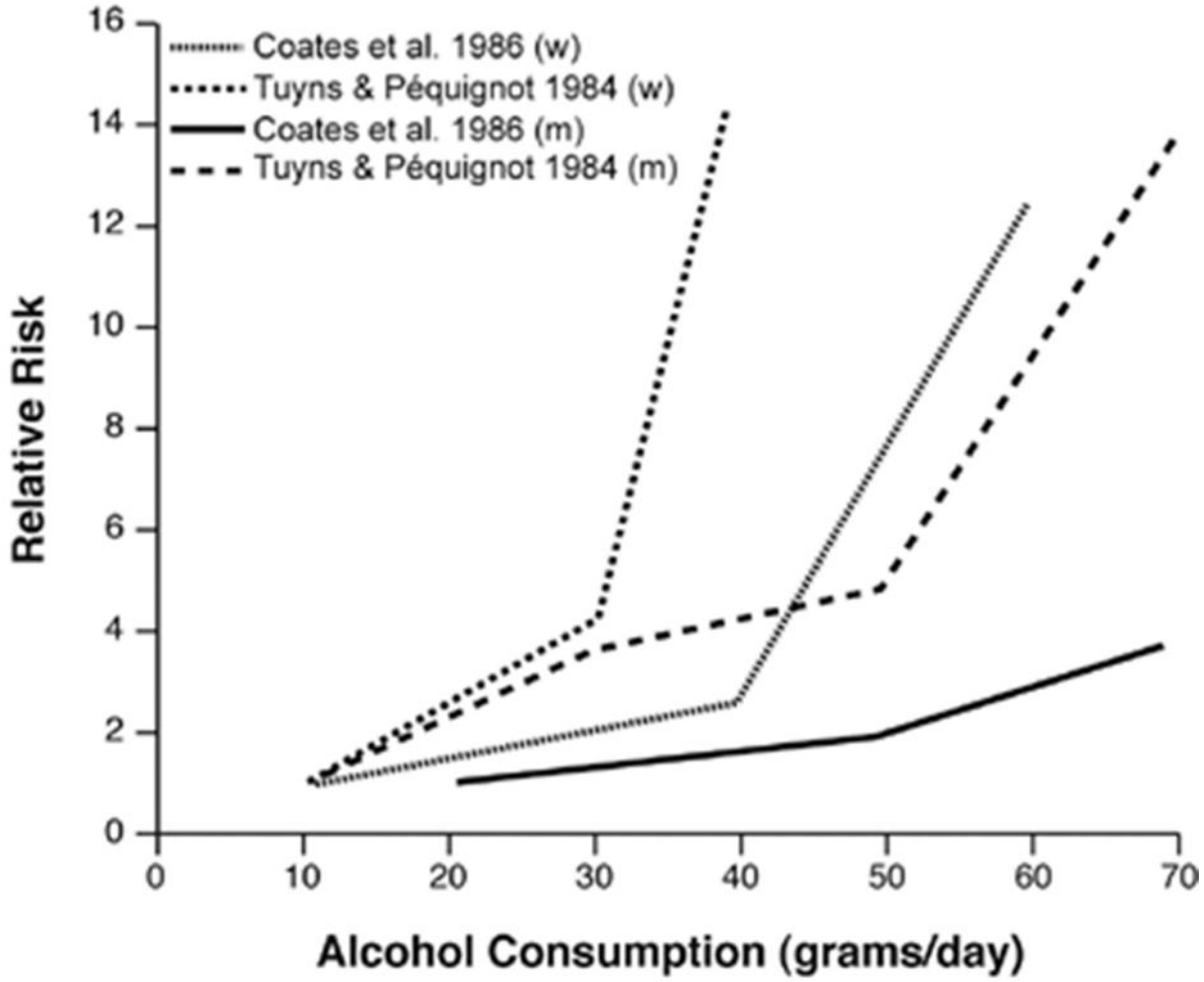
In 2010, total intake worldwide was 6.2 litres of pure alcohol per person of 15 years and above[4]

According to WHO ,total consumption by male drinkers 23.9 litres , female drinkers 10.4 litres of pure alcohol in India[5].Cirrhosis per 1 lakh population is 22.1%in male and 52.4% in female.

Global distribution of Daily Adjusted Life Years (DALY) by disease or injury is 9.6%[6].

The goal of WHO by year 2020 is to reduce mortality rate below 3.2 per 1 lakh population[6].

Cirrhosis Morbidity and Mortality and Average Alcohol Consumption



The strong link between heavy or excessive alcohol use and the development of liver disease took on added significance in the middle of the 20th century, when several researchers began exploring cirrhosis as a potential marker for levels of alcohol problems in populations Of particular importance was the discovery of a relationship between cirrhosis mortality rates and per capita levels of alcohol consumption in the population.

This relationship has proved to be remarkably strong and has been consistently observed across time periods and in various regions of the world European researchers have observed a lagged relationship between cirrhosis mortality and consumption measures, with the rate of cirrhosis mortality in a year being influenced by the alcohol consumption rates of several previous years

To account for this effect, Skog (1980) developed a “distributed lag model,” in which the effects of alcohol consumption in a year are distributed over the next several years. Using this model, he was able to explain an apparent inverse relationship between consumption and cirrhosis mortality rates in Great Britain between 1931 and 1958 (Popham 1970). Incorporating the distributed lag model into the data produced the expected positive relationship between consumption and cirrhosis mortality.

ANATOMY OF LIVER

The human liver constitutes 2% of adult body weight, it is around 1400 gms in females and 1800 gms in male.

Externally the liver is divided by falciform ligament into right and left lobes and attached to the anterior abdominal wall , through this ligament. It is a remnant of ligamentum teres ,which is vestigeal and recanalises during portal hypertension (PHT)[7].

Couinaud's Segmental Classification :

This is proposed for surgical resection purpose .Here liver is divided into anterior and posterior segment by the right hepatic vein .

Segment 1 –posterior caudate lobe.

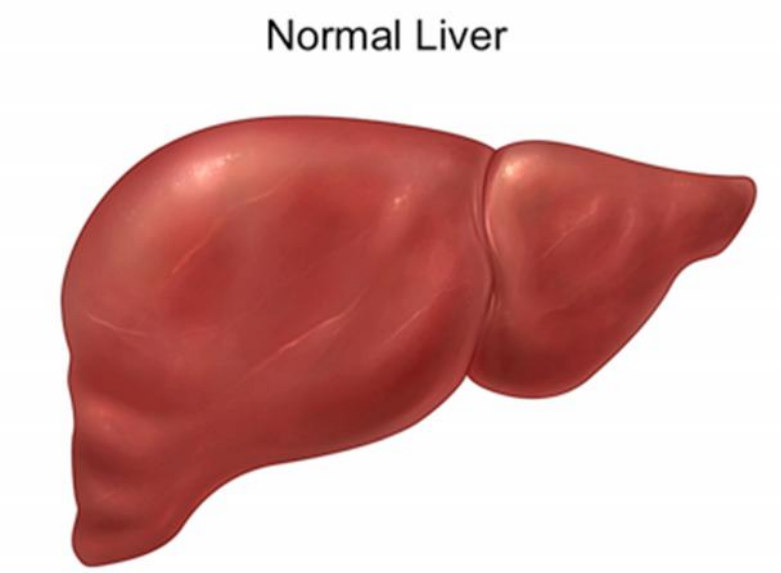
Segment 2 ,3 –left lateral

Segment 4-left medial

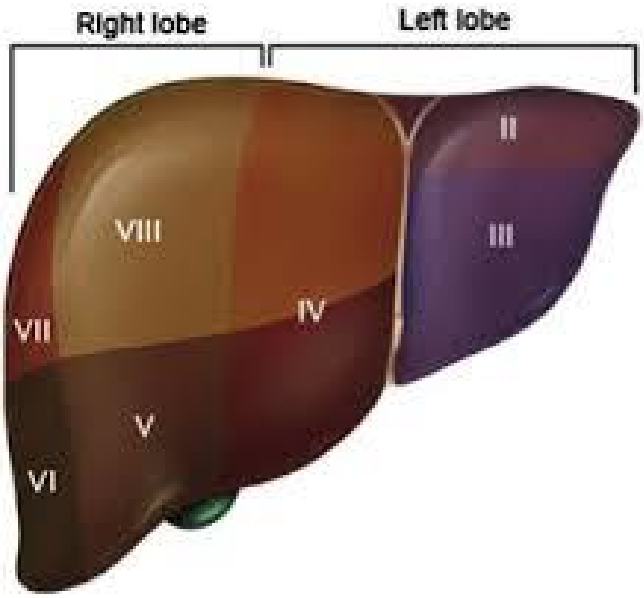
Segment 5,8 – right anterior

Segment 6,7 – right posterior.[7]

Diagram of liver

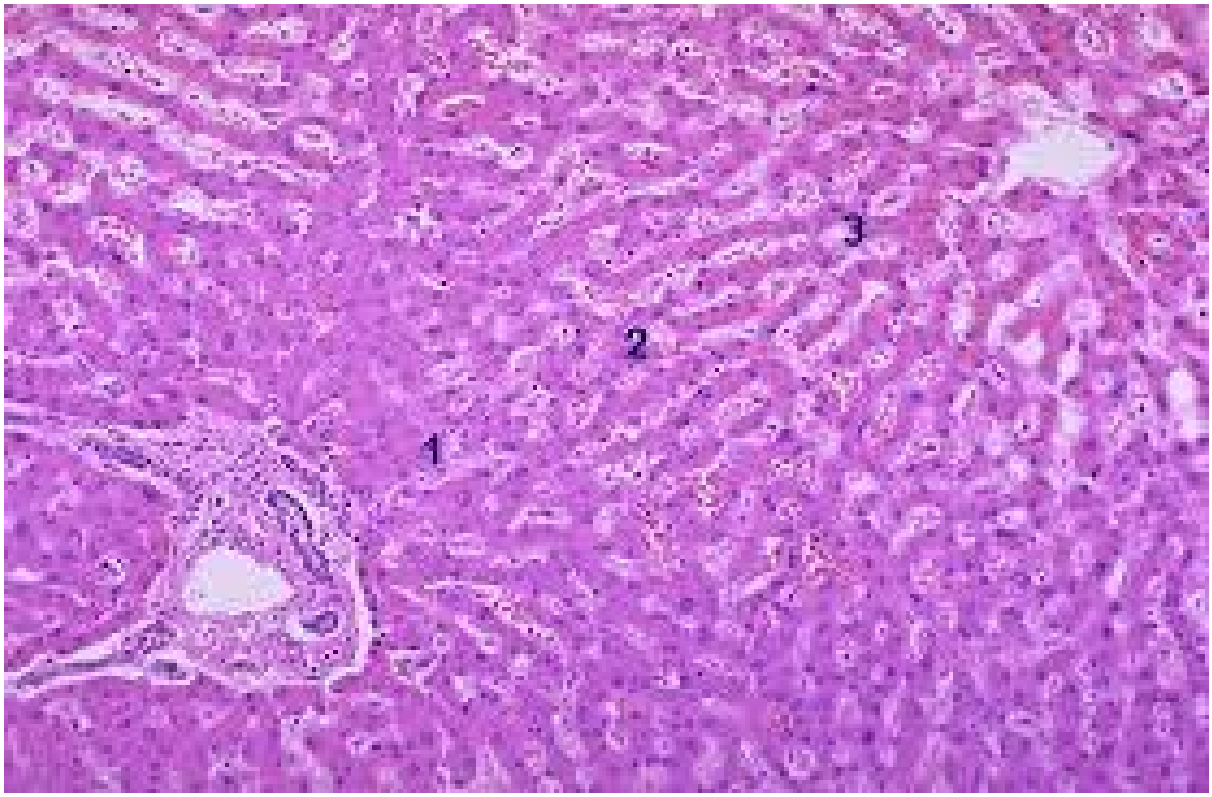


Segmental Anatomy



Arterial blood supply is mainly by portal vein (80%) and balance by hepatic artery(20%). Venous outflow is by 3 hepatic veins which directly drain into inferior vena cava. Lymphatics-The space of Disse and clefts of Mall produce lymph fluid. Liver has both parasympathetic and sympathetic nerve supply.[7]

Microscopy of hepatic system



Microarchitecture of liver:

The liver lobule consists of central vein, array of hexagonal hepatocytes, sinusoids and canaliculi carrying blood and bile. The periphery consist of the portal triad (hepatic artery, portal vein and bile duct).[8]

PHYSIOLOGY OF LIVER

Liver performs various functions like synthetic, storage, secretory, vascular, detoxification and metabolic function.

- Storage of glycogen, 90% retinol, Vit D, B12 and iron.
- Synthesis of ketone bodies, urea ,bile, plasma proteins, coagulation factors.
- Catabolism of heme , steroid and various drugs.
- Detoxification and modification of xenobiotic .
- Metabolism of carbohydrates, lipid and proteins[8].
- Phagocytic activity of liver is performed by kupffer cells present in the sinusoids[7].

ALCOHOL METABOLISM

A drink containing ethyl alcohol , when consumed becomes an alcoholic beverage. According to WHO the alcohol content necessary for the beverage, is the percentage pure alcohol by volume and it varies in different parts of the world like 0.7% in south east asia,1.1%in America,1.4 %in Europe[6].

It has a nutrient value of 7.1 Kcal/kg. Alcoholism is defined as repeated ingestion of alcohol with resultant dependency.

Alcohol is an agent which on chronic ingestion causes various conditions like

Hypoglycemia,

Wernickes-korsakoffspsychosis,

Ketoacidosis,

Gastritis,

Pncreatitis,

Cirrhosis liver,

Neuropathies,

Dementia,

Cardiomyopathy

Carcinoma of mouth ,larynx and oesophagus[9].

Absorption and Elimination

Gastro intestinal absorption of alcohol is by simple diffusion, 80% in duodenum and upper jejunum.

Elimination is by liver metabolism, certain amount by renal < 1 % and lungs 1.5 %.Lipid insoluble nature of alcohol leads to attain higher levels in obese body[9].

Metabolism:

Enzyme involved in alcohol metabolism are:

- **Alcohol dehydrogenase – major enzyme**
- **Isoenzyme of Cytochrome P-450, Microsomal ethanol oxidizing system – (MEOS)**
- **Catalase**
- **Xanthine oxidase**

Alcohol Dehydrogenase (ADH) system:

5 classes of ADH isoenzymes are present in humans which is encoded by different gene loci. Expression of genes of ADH are very specific.

Class I ADH activity is more in the liver with high affinity Vmax and low Km.

Class II only in the liver and

Class III in all the tissues.

Class IV in stomach.

In gastric mucosa the first pass metabolism of alcohol starts. The ADH present here has high affinity to alcohol and is higher in men , lesser in females and elderly[10].

In the liver the oxidizing capacity of the ethanol is maximum with ADH having very high affinity and low Km value.

$$CH_3CH_2OH + NAD^{+} \xrightarrow{ADH} NADH + H^{+} + CH_3CHO$$

Acetaldehyde is the metabolized product of ethanol by the action of alcohol de-hydrogenase enzyme.

The increase in the NADH causes the metabolic imbalance and acetaldehyde is the potent causative factor for alcohol induced pathogenesis in humans.

Alcohol metabolism undergoes zero order kinetics – 100 mg/ kg body weight / hour. Km of ADH is 4 mg/ 100 ml.

MEOS (Mitochondrial Ethanol Oxidising System) :

Km of MEOS is 50- 80 mg / 100 ml.

A small portion of alcohol is oxidized by cytochrome P450 – CYP2E1 the enzyme in endoplasmic reticulum which is induced by alcohol.

Catalase :

The enzyme, in peroxisomes takes part in less than 2 % of alcohol metabolism. It requires hydrogen peroxidase for its action[11].

Aldehyde dehydrogenase (ALDH):

This enzyme is coded by 4 independent genes on 4 different chromosomes. ALDH gene has two allelic forms ALDH 1 and ALDH 2. ALDH 2 has lower activity when compared to ALDH 1.

EFFECTS OF ALCOHOL

- Acetaldehyde is the major causative factor for the pathophysiology of alcoholic liver disease. Acetaldehyde forms adducts with many protein and impairs its function[12] .

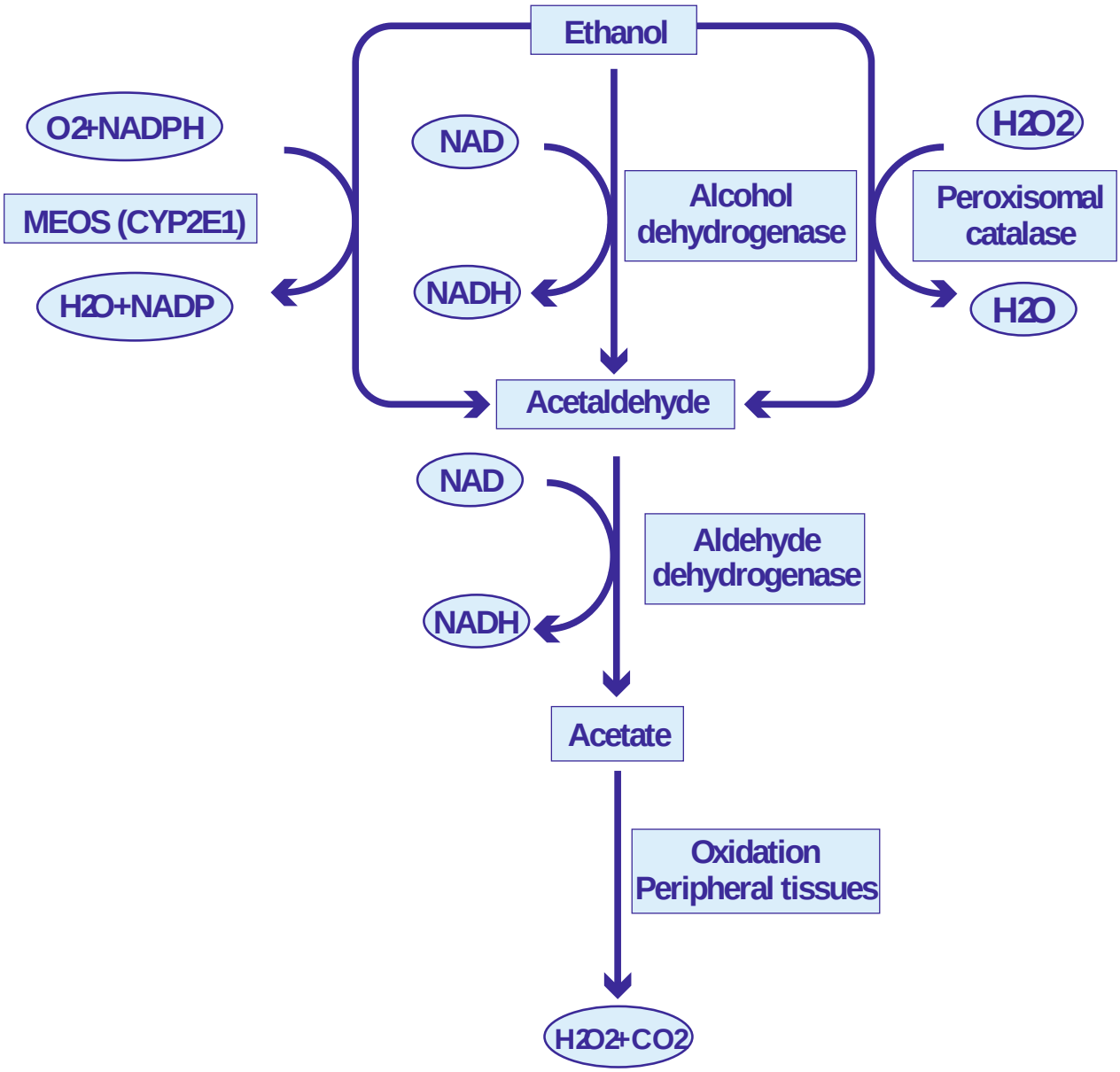
- The micro tubular function is disturbed due to the binding of acetaldehyde to alpha – tubulin .This leads to fat accumulation in golgi apparatus of perivenular hepatocytes.

- There is down regulation of microsomal triglyceride transfer protein (MTP) which does packaging of TAG (Triacylglycerols) and apo B into VLDL.

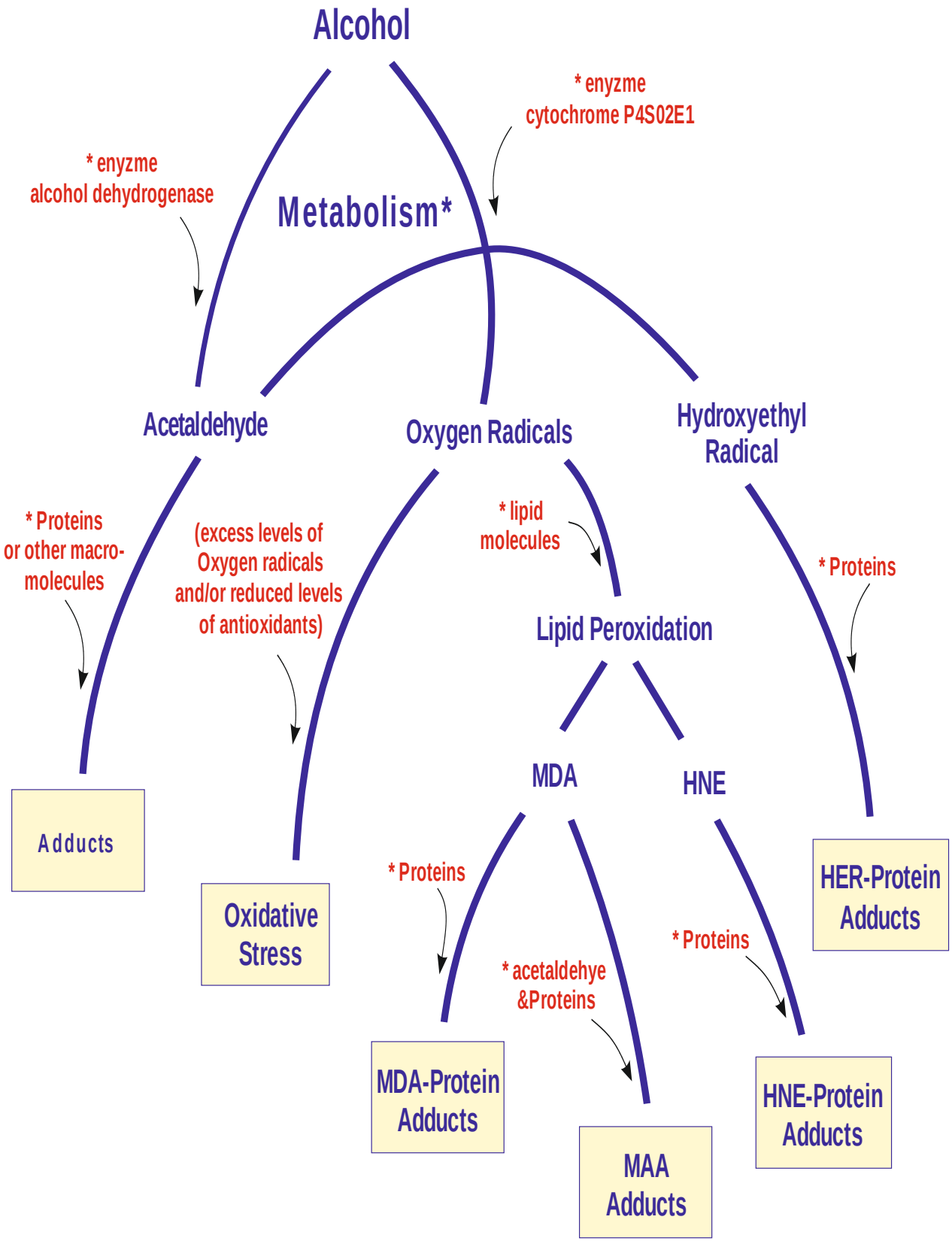
- Acetaldehyde inhibits PPAR – α ,a transcription factor, which regulates mitochondrial , microsomal and peroxisomal fatty acid oxidation systems in liver so that there is increase in free fatty acids in liver .

The overall effect of alcohol metabolism is the altered redox state, the increase in NADH/ NAD ratio which impairs gluconeogenesis , decrease in the substrate flow to TCA cycle ,inhibition of fatty acid oxidation and increase in TAG synthesis.

Hence alcohol acts as a potential hepatotoxin for the liver disease development depending on the existence of cofactors like gender, polymorphism of alcohol metabolizing enzymes, immunity, infection, nutrition and drug status[9].



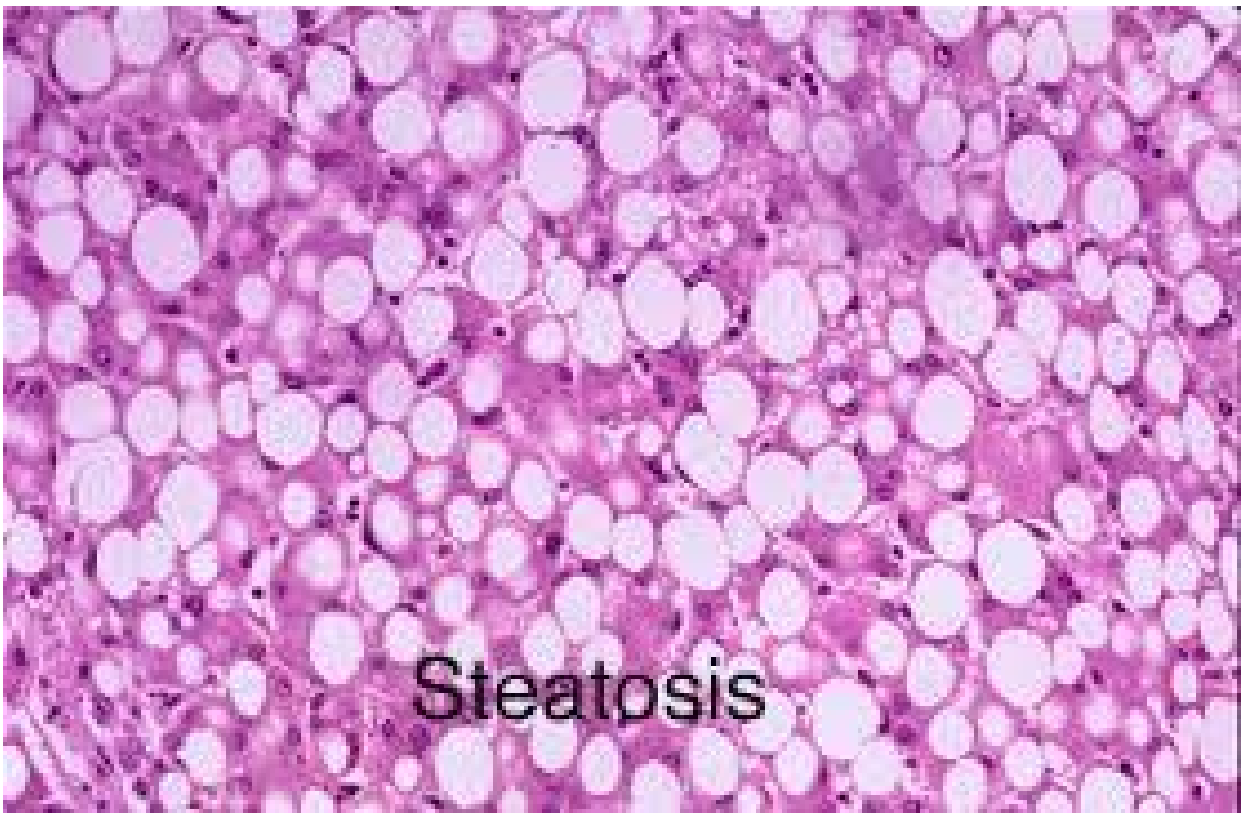
PATHOPHYSIOLOGY OF ALCOHOLIC DISEASE



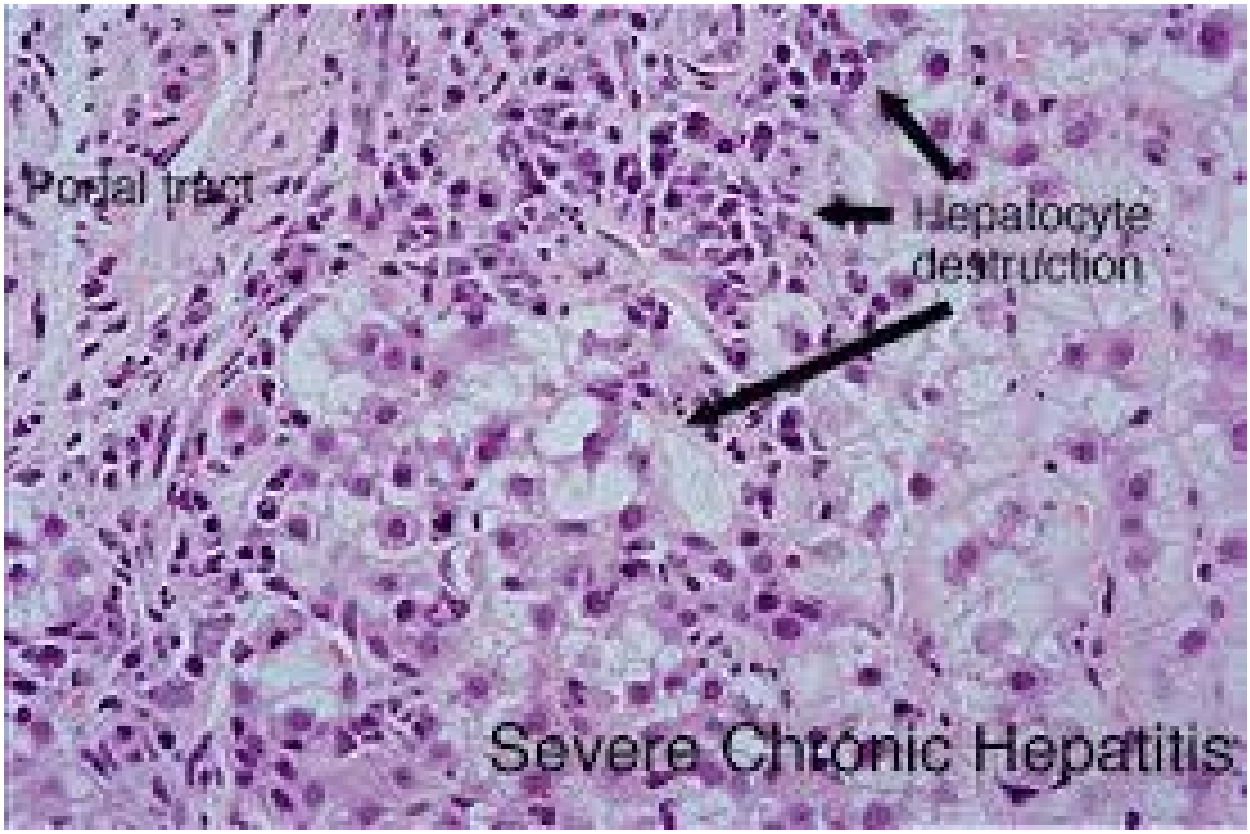
MORPHOLOGY OF ALCOHOLIC LIVER DISEASE

STAGES

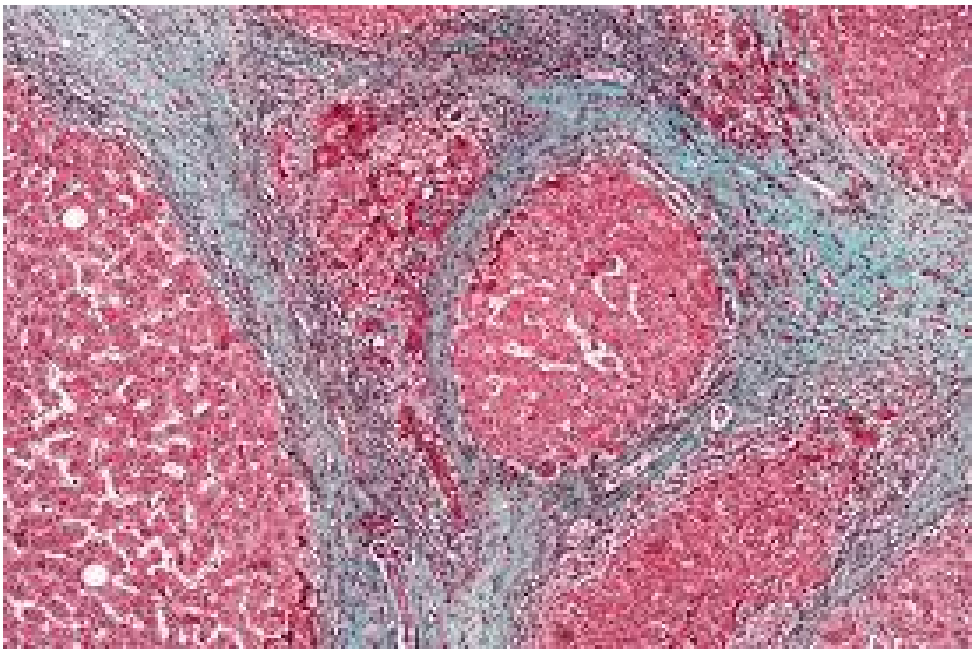
The first stage is the lipid droplet accumulation in the hepatocytes pushing the nucleus to the periphery of hepatocytes. Starting with centrilobular distribution it extends to the entire lobule.The fatty liver is large,soft yellow greasy organ now which is a completely reversal phenomenon on abstinence of alcohol[13].

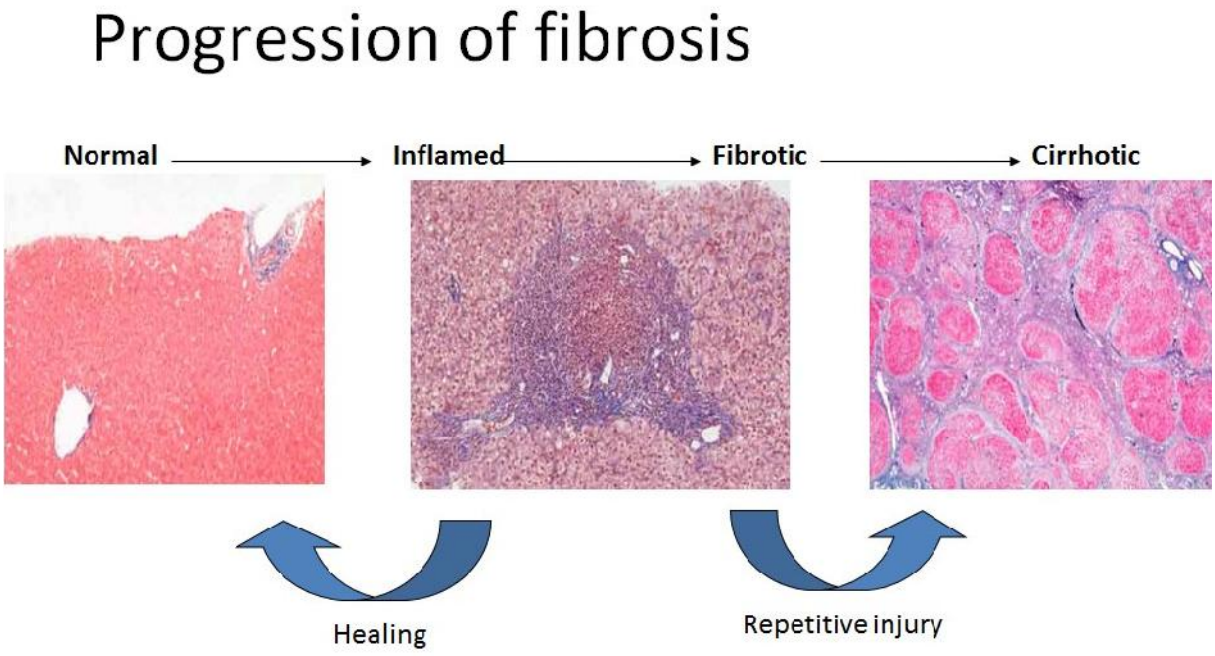


The second stage is the alcoholic hepatitis characterized by hepatocyte swelling, necrosis with mallory bodies. Neutrophilic infiltrations along with prominent activation of fibrosis which is due to stellate cell and portal tract fibroblast activation. Starting from perivenular region it extends on with repeated bouts of heavy alcohol intake. The liver is larger with visible nodules and fibrosis[13].



The final irreversible stage is evolution to cirrhosis. The cirrhotic liver is yellow tan, fatty and large during earlier stages transforming into shrunken non fatty organ at final stage. The fibrous septae are delicate and extend through sinusoids from central to portal regions[13].





MELD SCORE

Chronic liver failure leads to multi organ dysfunction and early death. Chronic alcoholism is one of the reason for chronic liver failure. Many studies have showed increased morbidity and mortality in patients with chronic liver failure undergoing surgical or interventional procedures[12].

In view of categorizing and predicting the outcome of such procedures various scoring systems were devised. Of which Child Pugh(CP) classification was more prevalently used. The parameters used were serum bilirubin, prothrombin time,serum albumin with two more clinical parameters like encephalopathy and ascites. Points were given for each indices and based on the cumulating of points risk, stratification is done. Even though CP classification is useful in cirrhotic patient undergoing medical management and as well predicting postoperative outcome it has been not sensitive in predicting short term outcome of morbidity (within 30 days) in cirrhotic individuals[12].

To overcome this deficit in scoring system MELD (Model for End-stage Liver disease) score was introduced. Initially it was used to predict morbidity outcome in patients with cirrhosis undergoing TIPS (Trans Jugular Intra Hepatic Porto systemic Shunt) procedure.

The MELD is a numerical scale ranging from 6(less ill) to 40(gravely ill) used for liver transplant candidates age 12 and older. It gives each person a score based on how urgently the patient needs the liver transplant within the next 3 months. The patients

score may go up or down over time, hence the score is assessed number of times while on waiting list.Status I category or Highly urgent patient (acute liver failure).

Three easily measurable values i.e. serum bilirubin, INR and serum creatinine are used. These values are entered in a formula and MELD score is arrived. Later MELD score was extended to measure mortality risk in hospitalized and ambulatory patients with cirrhosis. Its usefulness appears due to scoring irrespective of underlying disease etiology. In 2002 UNOS (United Network of Organ sharing) has introduced a modified MELD scoring system for organ allocation in patients with liver failure awaiting liver transplant[14].

The MELD score is calculated as follows :

MELD Score = 0.957 x Log e(creatinine mg/dL) + 0.378 x Log e(bilirubin mg/dL) + 1.120 x Log e(INR) + 0.6431

Multiply the score by 10 and round to the nearest whole number.

Laboratory values less than 1.0 are set to 1.0 for the purposes of the MELD score calculation.

The maximum serum creatinine value in MELD score is set to 4.0 and values are automatically interpreted according to recent dialysis.

MELD scoring is done for patients with age 12 and above. For patients with age less than 12 PELD score is used[14].

BIO CHEMISTRY OF ZINC

Zinc is one of the most essential trace element belonging to group 12 of the periodic table . It has a relative atomic mass of 65.38 and 30 as its atomic number, is found abundant in the human body[15,16].

It is considered to be a master hormone in the process of cell division and growth. The total adult body content is 2.25gms and distributed more in the muscle (55 %) followed by bone(30%) .The intake of zinc recommended daily is 14 mg per day for men and 9 mg for women .

Dietary sources : It is present in red meat , fish ,wheatgerm and wholebran in large quantities.

Absorption :

Absorption of zinc occurs throughout the intestinal tract mainly in jejujum. The efficiency of zinc absorption depends on the amount of intake. It is regulated by intake and as well by endogenous loss of zinc from intestinal fluids.

Transport of zinc [15,16]:

Zinc transportation is through significant transporters the ZiP(15) and ZnT(9).

ZiP type of transporters increase the intracellular concentration by promoting uptake while ZnT mobilises zinc out of cell. Zinc absorbed is taken by portal vein to liver

and is incorporated in metalloenzymes ,followed by plasma proteins.(80% to albumin and 20% to alpha -2 macroglobulin). Zinc content is high in prostate ,semen ,retina.

Excretion :

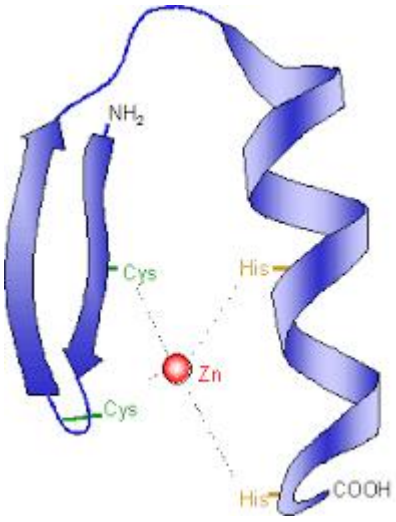
urine excretion per day is 0.5 mgs Faeces excretion per day is 10 mgs. The total intake equals the daily output.

Functions of zinc[15] :

It constitutes the major part of many enzymes like

- Aldehyde dehydrogenases
- Carbonic anhydrase
- Alkaline phosphatase
- RNA and DNA polymerases
- Thymidine kinase carboxy peptidases etc.,

- Zinc fingers :



The histidine and cysteine part of protein domain will have the tetrahedral zinc atoms and form zinc fingers and they have a role as transcription factors in developmental biology[16].

- Zinc has interaction with vitamin A in producing retinal binding protein and is involved in the activity of retinol dehydrogenase.
- Zinc is involved in ammonia metabolism, its deficiency impairs the function of urea cycle enzyme ornithine transcarbamylase.
- Zinc is very essential in taste acuity, normal membrane structure and function.
- Zinc is required for the maintenance of spermatogenesis and motility.
- Zinc plays a role in nucleic acid metabolism , synthesis of collagen hence it plays a important in wound healing.
- Zinc plays a role in innate and adaptive immunity .

- Zinc inhibits TNF –alpha activity and attenuates oxidative stress by reducing the production of reactive oxygen species.

Role of zinc in liver function[2]:

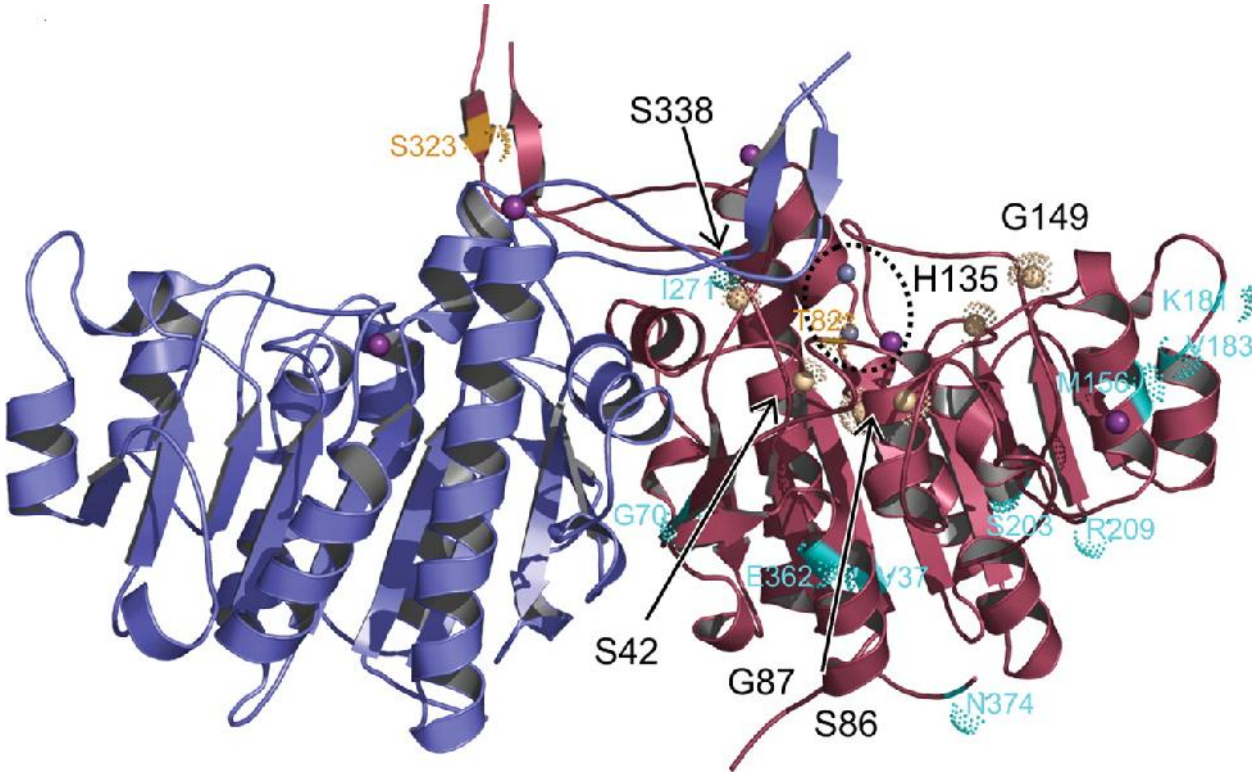
Zinc protects intestinal barrier function and prevents endotoxinemia, reduces pro-inflammatory cytokine production thereby preventing liver injury. Zinc suppresses alcohol elevated CYP 2E1 activity and increases ADH activity in liver. Zinc prevents alcohol induced decrease of GSH concentration and increases glutathione reductase activity thus suppressing alcohol induced oxidative stress.

Zinc attenuates alcoholic hepatitis and the m-RNA levels of TNF, FasL, FAF-1,Caspases,the factors associated with apoptosis of hepatocytes. Supplementation of zinc attenuates fibrotic changes. Hence zinc has wide functional ability in hepatoprotective mechanisms.

Laboratory assessment of zinc :

Sample recommended include fasting serum or plasma ,urine ,leukocytes and hair . A reference interval for serum zinc is 80 -120 µg/dl or 12 -18 µmol/L. For urine zinc 0.2 to 1.3 mg /24 h. Circadian changes include high values in the mornings than evening and post prandial decrease[17].

ALKALINE PHOSPHATASE



Alkaline Phosphatase E.C.No 3.1.3.1[18];Ortho phosphoric mono ester phosphohydrolase.

Optimum pH for activity is 8.4 -10

Activators : Mg^{2+} ,Co^{2+} and Mn^{2+}

Inhibitors : Citrate,Borate,Phosphate,Oxalate and EDTA.

Zinc is a constuent metal ion. The correct ratio of magnesium/zinc ions is necessary to attain optimal activity of the enzyme.

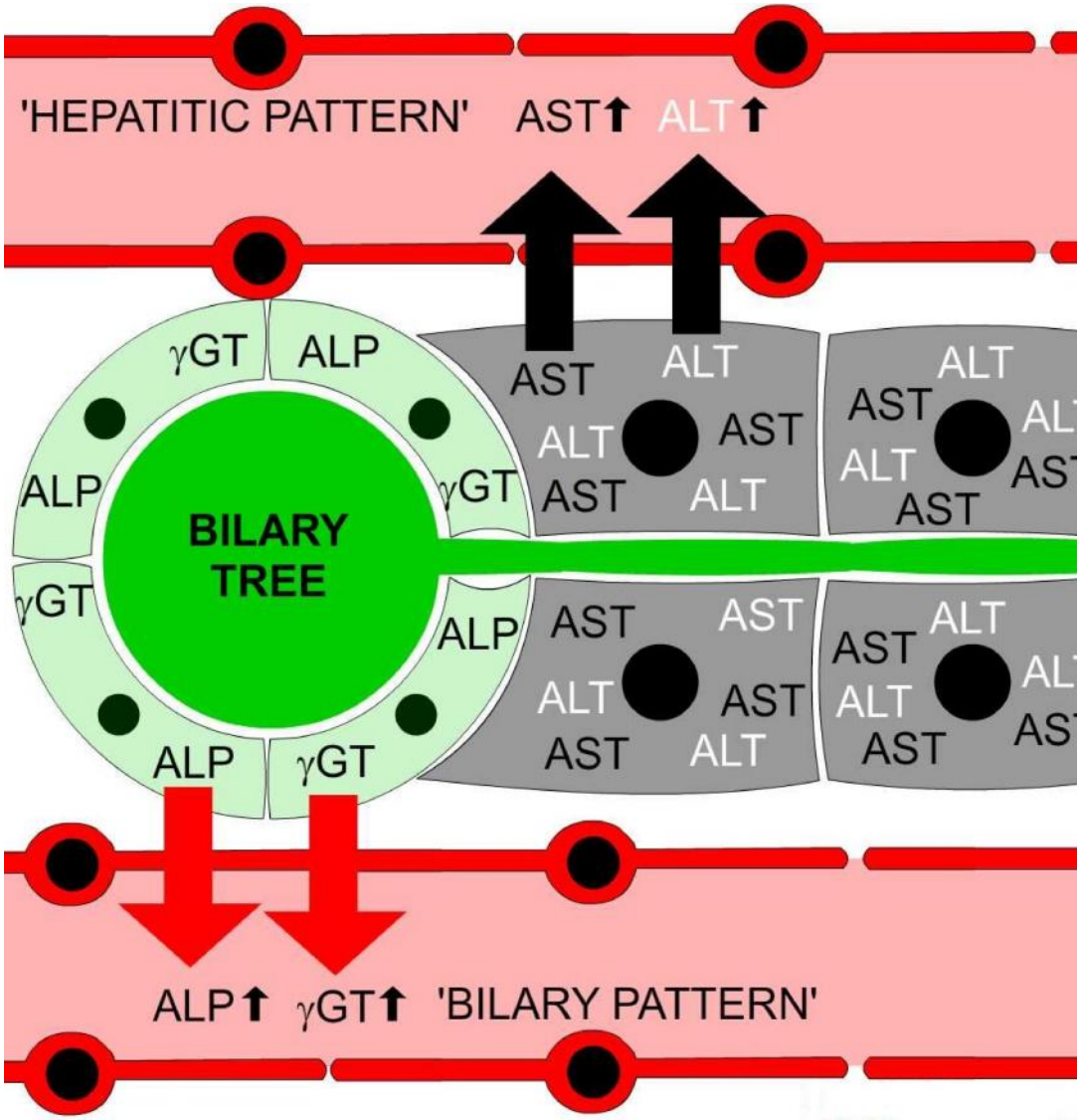
Alkaline phosphatase are group of enzymes that take part in hydrolysis of phosphates at higher pH. Contribution of this enzyme is mostly from liver, bone, placenta, least from intestinal epithelium and kidney[19].

Hepatic alkaline phosphatase is most densely represented near the canalicular membrane of the hepatocyte[19].The response of the liver to any form of biliary tree obstruction induces the synthesis of ALP by hepatocytes.

An experimental study shows one of the function of enzyme alkaline phosphatase in liver cell membrane is to hydrolyse phosphorylcholine and so that choline can cross canalicular membrane into the bile[20].

When cholestatsis happens due to intrahepatic origin bile secretion from hepatocyte to canaliculi is impeded which leads to regurgitation of the enzyme in to plasma which is reflected as increase activity[21].

Increased activity of alkaline phosphatase with increased γ- GT in serum almost always reflects etiology of hepatobiliary origin[21].



Accordingly, diseases that predominately affect hepatocyte secretion (e.g., obstructive diseases) will be accompanied by elevations of alkaline phosphatase levels. Bile-duct obstruction, primary sclerosing cholangitis, and primary biliary cirrhosis (PBC) are some examples of diseases in which elevated alkaline phosphatase levels are often predominant over transaminase level elevations[22].

REVIEW OF JOURNALS

Craig Mc Clain et al, Annals of Hepatology (1) 2008,5-15.

The author delivers a concise review about the treatment of alcoholic liver disease. The review states that nutritional supplementation is a benefit along with abstinence from alcohol, smoking and weight loss. Pentoxiphylline being beneficial in alcoholic hepatitis. Complementary agents like zinc,milk,SAM have great therapeutic rationale. Liver transplantation is the only solution for end stage liver disease[23].

- **Ferdousi,ahia et al,Journal of Medical Biochemistry 2012(5(2)) 44-47.**

The study evaluates the zinc status in patient with liver cirrhosis.The study has shown a significant lower plasma zinc level in cirrhotic patients.When compared with normal controls estimated by colorimetry the age and gender related bias were prevented by taking in to account the similar range of age[24].

- **Ana.C.R Schneider et al,Journal de Pediatria 2009 , 85-04/359-364**

The study has been conducted to assess zinc status in paediatric cirrhotic patients. The author has investigated the association results obtained from atomic absorption spectro photometry. Anthropometric measures have also been included to relate to the level of zinc.The results were prevelance of hypozincemia in 43% of patients with cirrhosis.There is no association between anthropometric levels and zinc levels[25].

- **Y.Takumme,et al Alimentary pharmacology and therapeutics – 2010: 32: 1080-1090.**

This is a clinical trial where the study has evaluated the changes in the physical component scale and mental component scale of alcoholic patients with HE and control population. The author has also concluded that zinc supplementation has decreased HE grade , blood ammonia levels and improved CP scale. It was not significantly associated with changes in MCS[26].

- **Krenitsksy et al ,nutrition issues in gastroenterology series 6, June 2003**

The article address certain strategies to overcome hurdles in the nutritional delivery in the population of hepatic failure .The author has stated the significance of zinc in the function of liver in detoxifying and improving encephalopathy scores[27].

- **Kaushikkar, et al Indian Medical Gazzete–feb 2013/74-78**

The author has analysed serum zinc and albumin levels in cirrhotic patients and controls and has stated a significant decreased in zinc and albumin with significant P value <0 .001[28].

- **Mohammad zhou et al nutrition in clinical practice 2012.**

The article states about zinc and its significance in liver function, manifestations of its deficiency and emphasizes on zinc supplementation to stabilize gut barrier function ,decreasing the endotoxins and oxidative stress[2].

- **Yang et al,1991 June;22(2);201-3**

The author introduces a micro method for zinc and copper estimation by concentrating peripheral blood by wave oscillopolarography. The author states the method to be superior and simple compared to atomic absorption spectrophotometry[29].

- **Makino et al Clin .ChemActa 1982 mar 26;120(1);127-135**

The author introduces a colorimetric method for accurate determination of serum zinc .The within day precision (cv) are in range of 0.3 to 3.5% and 1.9 -3.1 %.The author had obtained a good correlation between colorimetry and AAS[30].

- **Makino et al Clin Chem Acta 1999 Apr ;282 (1-2)65 -76**

A simple and sensitive assay of zinc in serum using cationic porpyrin .The author has introduced a method that has a cationic porphyrin (4-N tri methyl amino phenyl porphinetetratoluenesulphonate salt .7 iodo 8 hydroxyl quinolone 5 sulphonic acid (ferron)as a accelerator[31].

- **Beckett et al Ann Clin Biochem 2009 July ;46 322-6**

The author has made a comparison between two method calorimetric and AAS and has concluded that the results have systematic and fixed bias and hence AAS to be superior for determination of copper and zinc[32] .

- **Arnand j et al Clinbiochem 1993 trial on determination of copper and zinc feb;26(1)**

The author has conducted a inter-laboratory trial on determination of copper and zinc by two different methods.The author states sensitivity and linear range to be good in calorimetry and also correlated satisfactorily[33].

- **Patrick et al Hepatology 2007;45;797-805**

The author in this article reviews the MELD score, compares with other scores and depicts the strength and limitations of the score .He also suggests for right method of analysis of various objective variables taken into account. He suggests for better refinement of the score in future[34].

- **Azam hyder et al European journal of experimental biology 2013,3(2)280-284**

The author has analysed various enzyme panels in liver disease by standard methods and has concluded that AST,ALT,ALP,GGT were significantly raised in patients than in controls with significant p' value(<0.001)[35].

- **Kareweissman et al the American journal of clinical nutrition june 1985/ 1214-1219**

The author has analysed serum zinc and ALP during zn supplementation in patients with proven zinc deficiency and elderly and normal controls. Zinc and ALP levels rose after zinc therapy initiation ,decreased after stoppage . Hence he has suggested serial serum Zn and ALP estimations could be a valid tool in diagnosis of zinc deficiency[36].

- **Xinqinkang et al Hepatologyoct 2009 Vol 50/1241-51**

The author has done an elaborative study molecular diagnostic wise using PCR immunoblotting and has stated that zn supplementation normalizes alcoholic steatosis and reactivates TNF –alpha and PPAR –alpha on zinc availability and oxidative stress[37].

- **Han-chieh Lin et al Liver transplant 12;65-71 2006**

The author has proposed a modified CTP score and compares to be better than original one and MELD score[38].

- **J.C.Smith et al ClinChem 25/8 1478-1491(1979)**

The author has concluded the direct dilution method when compared to calorimetry and induction coupled plasma emission to be accurate ,precise ,free of specific iron interference and simple[39].

- **Kuldipsingh et al international journal of research in health science Oct - Dec 2013- Vol 3 issue 1**

The author has illustrated the various liver function tests under various sub heads and established the need for the whole profile of LFT in liver diseases[40].

- **Matsuoka et al, Journal of Clinical Biochemistry.Nutrition., 45,Page 292-303,Nov 2009**

The study is about the effect of zinc and BCAA granules supplementation especially in HCV related carcinogenesis of liver[41].

MATERIALS AND METHODOLOGY

This is a case control study conducted during the period from january 2015 to june 2015.Prior approval was obtained from the Institutions Ethical Committee.

The study involves two groups, Group – I with 50 healthy members as control, recruited from master health checkup at Stanley Medical College and neighborhood.

Group – II with 50 cases of pre-diagnosed alcoholic liver disease from Medical Gastro Enterology Department, Stanley Medical College Hospital.

Written consent with explained protocol was obtained from both groups .

Inclusion criteria :

Patients diagnosed to have ethanol related decompensated liver disease with or without portal hypertension.

Exclusion criteria :

- Patient with non-alcoholic liver disease.
- Patients on zinc supplementation.
- Patients with malabsorption syndromes.
- Patients who had undergone intestinal resection procedures.
- Patients with co-morbid conditions like diabetes mellitus, malignancy, renal failure, known viral disease and on treatment with steroids, hormones.

Sample Collection and Preparation:

5 ml of venous blood from ante-cubital vein (over night fasting sample) was collected under strict aseptic precautions. Serum samples were separated by centrifugation at 2000-2500 rpm for I5 minutes and used for analysis. Samples were stored at -20 ^{0}C until analysis.

Estimation of Zinc :

Colorimetric method – end point

Principle :

Zinc forms a colored complex when reacting with a specific complexant 5 – Br-PAPS. The intensity of color formed is proportional to the amount of zinc present in the sample, measured at 560 nm

Sample :

Serum,plasma ,seminal fluid and urine .

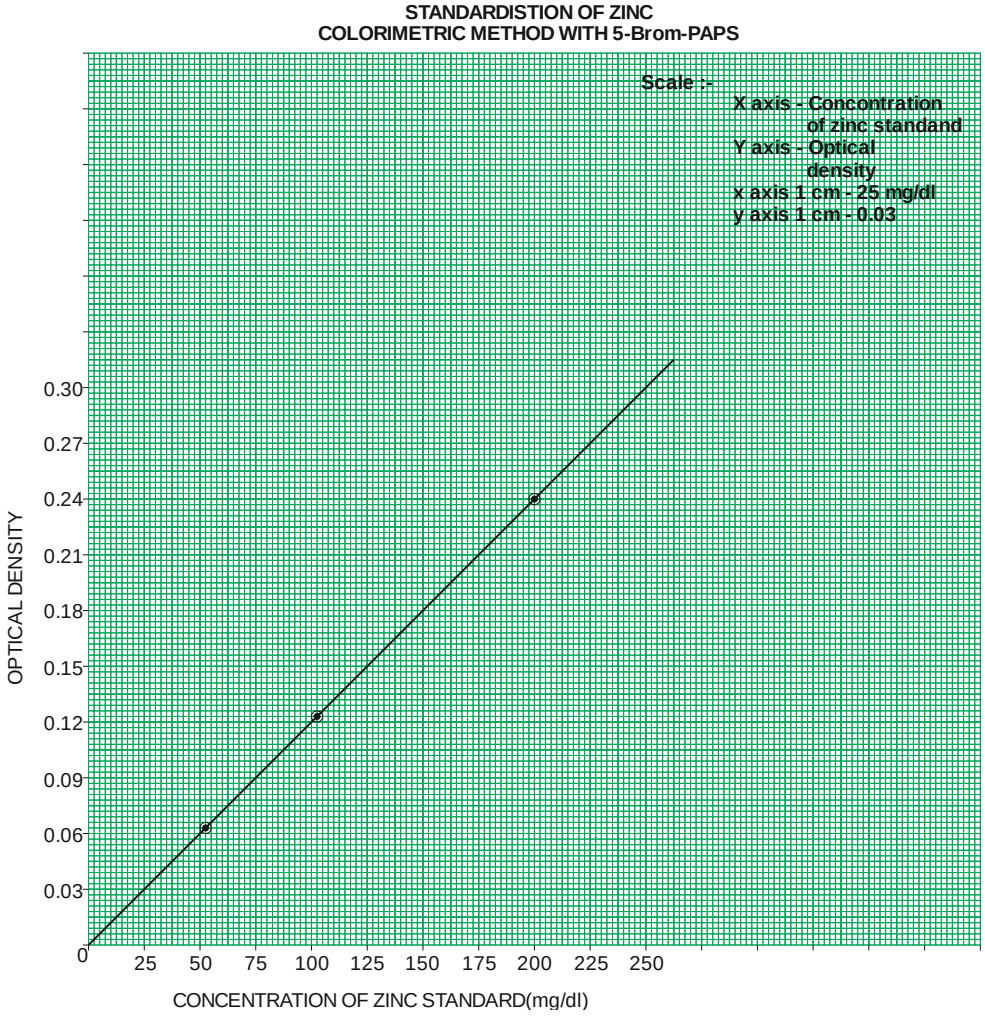
Reagents :

R1	Buffer Good pH 8.6	0.2 mol/L
R2	Color 5- bromo PAPS	1.1mmol/l
R3	Reducing substance ascorbic acid	

ABSORBANCE OF ZINC STANDARDS

Reagent Blank = 0.105

Concentration of zinc(µg/dL)	Absorbance	Standard – blank Absorbance
50	0.166	0.061
100	0.227	0.122
200	0.345	0.240



Calibration : The zinc calibrator value is verified by NIST traceable standard.

Zinc calibrator 200 µg

Preparation :

Working reagent: R3 is mixed with r1.mix well stable for 30 days at 2-8 days.

Procedure:

Reagents at room temperature

	Blank	Standard	Sample
Working reagent	1.0	1.0	1.0
Distilled water	50	-	-
Standard	-	50	-
Sample	-	-	50

Mix and absorbance is read at 560nm against blank.(A1)

	Blank	Standard	Sample
R2	100	100	100

Mix and read the absorbance of sample (A2).

Calculation :

(A2-A1)/(A2-A1) X 200 = CONC of ZINC

Conversion factor : µg/dl X 0.153 = µmol/L

Reference valves:

Serum - 68-107 µg/dl

Centrifuged seminal fluid – 2-10 mg/dl

24 hrs – urine 150-1200µg/L

Measuring range : 4µg/dl – 2000 µg/dl

ESTIMATION OF CREATININE

Serum creatinine is more specific and sensitive indicator of renal function along with urea .

Principle :

Creatinine + H_2O ⟶ Creatine

Creatine+ H_2O ⟶ Sarcosine + Urea

Sarcosine + O_2+ H_2O ⟶ Glycine + HCHO+4 H_2O_2

$2H_2O_2$ + 4 –AA+ TOOS ⟶ Quinone pigment +4 H_2O

Reagent :

Creatinine R1

Creatinase ,Sarcosine oxidase,TOOS

Creatinine R2

Creatinase

Peroxidase

4-AA

Creatinine standard concentration-2 mg/dl

The reagent is linear up to 200 mg/dl

Normal range :

Serum Male : 0.6 – 1.1 mg/dl

Female : 0.5 – 0.8mg/dl

Urine Male : 1070-2150 mg/ dl

Female : 769 – 1200 mg/dl

System parameters:

Mode of reaction	Endpoint
Slope of reaction	increasing
Primary wavelength	546nm

Secondary wavelength	630nm
Temperature	37°C
Std conc .	2mg/dl
Linearity	200 mg/dl
Blank	Reagent
Incubation time	10 min
Sample vol	10 µl
R1	450µL
R2	150 µL
Cuvette	1 cm light path

Procedure:

	Blank	Calibrator	Sample
Reagent µl	450	450	450
Calibration		10µl	
Sample			10µl

Mix and incubate for 5 min at 37 °C. Measure the absorbance.

Calculation :

Creatinine concentration = (Abs of sample /Abs of standard) X Std concentration

ESTIMATION OF PROTHROMBIN TIME

The prothrombin time is an indicator of the extrinsic pathway of coagulation cascade.

Principle :

Tissue thromboplastin in the presence of calcium activates extrinsic pathway of human blood clotting system. When thromboplastin reagent is added to citrated plasma , clotting is initiated and clot forms. Activation time is proportional to the concentration of clotting factors.

Reagent :

Tissue factor (rabbit origin)

Calcium

Preservative

Stabilizers

Pack contents:

PT reagentsR1

Buffered trisodium citrate (0.109)

Normal range :

Prothrombin time - 11—15 sec

INR value – 0.8 – 1.5

Sample collection :

Citrated plasma,mix gently 9 parts blood and 1 part of 3.2% trisodium citrate , centrifuge for 15 min at 3000 rpm to get platelet free plasma .

Procedure:

Pre warm the reagent at 37 c for 10 min

Pipette 100µl of plasma incubate for 3 min

Add 200µl and simultaneously start timer and watch for clotting to occur, note time immediately, it is the prothrombin time .

Turbid ,lipaemic and hemolysed samples are to be avoided.

ESTIMATION OF BILIRUBIN

METHODOLOGY : Diazo method by Lee and Pearlman

Principle :

Sulphanilic acid reacts with sodium nitrate to form diazotized sulphanilic acid. Total bilirubin reacts with diazotized sulphanilic acid in the presence of DMSO to form azobilirubin.

Reagent Composition:

Total bilirubin reagent	-	2 x 50 ml
Sulphanilic acid	-	28.9mol/L
HCl	-	165mmol/L
DMSO	-	7mmol/L
Total Bilirubin Activator	-	1x4ml
Direct bilirubin agent	-	2x50ml
Sulphanilic acid	-	28.9mmol/L
HCl	-	165mmol/L

Direct Bilirubin activator - 1x4ml

Bilirubin artificial standard - 1x4ml

Total bilirubin standard concentration - 10mg/dl

Direct bilirubin standard concentration - 7.5mg/dl

Storage and Stability :

The reagents are stable when stored at right temperature.

The standard and activator should be stored at 2-8^0 C.

Linearity:

This reagent is linear up to 20mg/dL.If the concentration is greater than linearity (20mg/dl) dilute the sample with normal saline .

Multiply the result with dilution fraction.

Normal range :

It is recommended that each laboratory establish its own reference values.

The following value may be used as guideline.

Total bilirubin up to 1.2mg/dl

Direct bilirubin up to 0.4mg/dl.

Preparation and Stability of Reagent :

Reagents are ready to use.

Avoid direct exposure of reagent to light.

Sample:

Serum/Plasma (without hemolysis)

SYSTEM PARAMETER

Mode of Reaction	End point
Slope of Reaction	Increasing
Wavelength	546-532 nm
Temperature	30^0C
Factor(Total)	20.5
Factor(Direct)	16
Blank	Sample blank
Linearity	20 mg/dl
Reaction time	5 mins

Sample volume	50 µL
Reagent volume	1000µL
Activator	20µL
Cuvette	1 cm light path

LABORATORY PROCEDURE

	Total Bilirubin		Direct Bilirubin	
	Sample blank	Test	Sample blank	Test
T.Bilirubin reagent	1000µL	1000µL	-	-
D.Bilirubin reagent	-	-	1000µL	1000µL
Activator(T/D)	-	20µL	-	20µL
Serum	50µL	50µL	50µL	50µL

Mix well and incubate for 5 minutes. Measure the absorbance of the sample against sample blank at 546 or 532 nm.

CALCULATION:

Total Bilirubin =OD of test-od of sample blank x Factor

Direct Bilirubin =OD of test-od of sample blank x Factor

With artificial standar

Total bilirubin Concentration = $\frac{OD\ of\ test - OD\ of\ blank}{OD\ of\ standard - OD\ of\ blank} \times 10$

Direct bilirubin Concentration = $\frac{OD\ of\ test - OD\ of\ blank}{OD\ of\ standard - OD\ of\ blank} \times 10$

ESTIMATION OF GAMMA –GLUMATYL TRANSFERASE (GGT),

IFCC Method ,Kinetic

Methodology:

The GGT-Soluble substrate method(GGT-SS), GGT present in the sample transfers the glutamyl group from the substrate to glycylglycine to form glutamyl glycylglycine and 5-amino-2-nitrobenzoate.

Principle:

L - γ - glutamyl – 3 – carboxy – 4 – nitranilide + Glycylglycine

GGT

L - γ - Glutamyl glycylglycine + 5 – amino - 2 – nitrobenzoate

REAGENT COMPOSITION

REAGENT 1 : GGT Reagent

ACTIVE INGREDIENTS	CONCENTRATION IN THE TEST
Tris buffer(pH8.2±0.1 at 20^0C	100 mmol/L
Glycylglycine	100 mmol/L
L - γ - glutamyl – 3 – carboxy – 4 – nitranilide	2.9 mmol/L

REAGENT RECONSTITUTION

Allow the reagent bottle and Aqua-4 to attain the room temperature15-30^0C.Add the amount of Aqua-4 contents of each vial. Swirl to dissolve

The reagent is stable for at least 21 days when stored at 2-8^0C or for 5 days at room temperature of 15-30^0C. Discard with reagent greater than 0.7 at 405nm(1 cm) when read against a distilled water blank.

SAMPLE :

Unhemolysed serum and plasma (Heparin or EDTA) are suitable for use with this method.Anticoagulants like EDTA will interfere with the assay and hence not be used.

GGT is stable for 7 days when stored at 2-8^{0}C.

ASSAY PARAMETERS:

Mode	Kinetic
Wavelength(nm)	405
Sample Volume(µl)	50/100
Reagent Volume(µl)	500/1000
Lag time(sec)	30
Kinetic interval(sec)	60
No. of readings	3
Kinetic factor	1158
Reaction Temperature(^{0}C)	37
Reaction Duration	Increasing

Normal Low(IU/L)	0
Normal High(IU/L)	50
Linearity Low(IU/L)	0
Linearity High(IU/L)	450
Absorbance(Max)	0.700
Blank with	Water
Units	IU/L

ASSAY PROCEDURE

	Volumes
Working reagent	1000µl
Test	100µl

CALCULATION

The general formula for converting absorbance change into International Units (IU) of activity is

$$IU = \frac{(\Delta A/min)\ X\ T.V\ X\ 10^3}{S.V\ X\ Absorptivity\ X\ P}$$

T.V = total reaction volume in µl

S.V = sample volume in µl

Absorptivity = millimolar absorptivity of L- γ -glutamyl – 3 – carboxy – 4 – nitroanilide 405 nm = 9.5

P = cuvette lightpath (cm) = 1 cm

Activity of GGT at 37^0C (IU/L) = (ΔA_{405} / min) X Factor (1158)

LINEARITY

Upto 450 IU/L

NORMAL VALUES (Reference for guidelines)

TEMPERATURE	MALE	FEMALE
37^0C	≤ 50 U/L	≤ 30 U/L
30^0C	≤ 39 U/L	≤23 U/L
25^0C	≤ 17 U/L	≤ 17 U/L

PERFORMANCE DATA

PrecisionWithin run

	LEVEL I	LEVEL II
Number of samples(n)	20	20
Mean	20	67
S.D	0.41	1.00
C.V(%)	2.05	1.49
Between run		
Number of samples(n)	20	20
Mean	22	67
S.D	1.80	2.05
C.V(%)	8.18	3.06

ESTIMATION OF ALBUMIN

Method : BCG

TEST PRINCIPLE

In the presence of bromocresol green at a slightly acidic pH serum albumin produces a color change of the indicator from yellow-green to green-blue. The intensity of the blue-green color is proportional to the concentration of albumin in the sample.

TEST PARAMETERS

Method	Colorimetric, Endpoint, Increasing reaction, BCG
Wavelength	546 nm
Temperature	37^0C
Sample	Serum, heparin or EDTA
Linearity	Up to 6 g/dL
Sensitivity	The lower limit of detection is 0.2 g/dL

REAGENT COMPOSITION

Citrate buffer pH 4.2	30 mmol/L
Bromocresol green	0.26 mmol/L

MANUAL TEST PROCEDURE

	Blank	Std./Cal.	Sample
Reagent	1000µL	1000µL	1000µL
Sample	-	-	10µL
Std./cal.	-	10µL	-
Dist.water	10µL	-	-

Mix, Incubate for approx. 10 min at 20-25^{0}C/37^{0}C and read absorbance against reagent blank within 60 min.

CALCULATION

$$\text{Albumin (g/dL)} = \frac{\Delta \text{A Sample}}{\Delta \text{A Std/Cal}} \text{ X Conc.of Std/Cal (g/dL)}$$

REFERENCE RANGE

	g/dL	µmol/L
Adults	3.5 – 5.2	507 – 756

ESTIMATION OF SGOT

Method :

IFCC method

Optimized UV test according to IFCC

Principle

L-Aspartate + 2-Oxoglutarate $\xleftrightarrow{\text{ASAT}}$ L-Glutamate + Oxaloacetate

Oxaloacetate + APADH(NADH analogue) + H^+ $\xleftrightarrow{\text{MDH}}$ L-Malate + APAD (Oxidized NADH analogue)

Reagents

R1	TRIS pH 7.65	100 mol/l
	L-Aspartate	250 mmol/l
	MDH(Malate Dehydrogenase)	≥ 550 U/l
	LDH (Lactate Dehydrogenase)	≥ 700 U/l
R2	2- Oxoglutarate	10 mmol/l
	APADH(NADH analogue)	0.20 mmol/l

Mix R1 and R2 (800 µl R1 + 200 µl R2) along with sample at the time of testing.

Specimen

Unhemolysed freshly collected serum/ EDTA plasma (morning samples are preferred)

Test Procedure

Take the following in a clean glass test tube

R1	0.8 ml
R2	0.2 ml
Serum/Plasma	100 µl

Mix well and after 60 seconds incubation, measure the change in optical density per 60 seconds during 180 seconds against distilled water at 340 nm as follows

A_0 - Exactly after 60 seconds

A_1, A_2, A_3 - Exactly after every 60 seconds for 180 seconds

Activity of SGOT in IU/L

At 340 nm in IU/L = Abs/min x 1975

System Parameters

Reaction type	Kinetic
Reaction Direction	Decreasing
Wavelength	340 nm
Flow cell temp	37^0C
Zero setting with	Distilled water
Delay time	60 secs
Kinetic interval	60 secs
No.of readings	4
Reagent volume	800 µl R1 + 20 µl R2
Sample volume	100 µl
Factor	1975
Linearity	500
Units	IU/L
Low normal	0.00
High normal	40.0

ESTIMATION OF SGPT

IFCC method

Method :

Optimized UV test according to IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) Modified

Principle

L-Aspartate + 2-Oxoglutarate $\xleftrightarrow{ALAT}$ L-Glutamate + Pyruate

Pyruate + APADH(NADH analogue) + H^+ $\xleftrightarrow{MDH}$ L-Lactate + APAD (Oxidized NADH analogue)

Reagents

R1	TRIS pH 7.5	250 mol/l
	L-Alanine	500 mmol/l
	LDH (Lactate Dehydrogenase)	≥ 5000 U/l
R2	2- Oxoglutarate	20 mmol/l
	APADH(NADH analogue)	0.25 mmol/l
	Azide	0.1%

Mix R1 and R2 (800 µl R1 + 200 µl R2) along withsample at the time of testing.

Specimen

Unhemolysed freshly collected serum/ EDTA plasma (morning samples are preferred)

Test Procedure

Take the following in a clean glass test tube

R1	0.8 ml
R2	0.2 ml
Serum/Plasma	100 µl

Mix well and after 60 seconds incubation, measure the change in optical density per 60 seconds during 240 seconds against distilled water at 340 nm as follows

A_0 - Exactly after 60 seconds

A_1, A_2, A_3 - Exactly after every 60 seconds for 240 seconds

Activity of SGPT(ALT) in IU/L

At 340 nm in IU/L = Abs/min x 2225

System Parameters

Reaction type	Kinetic
Reaction Direction	Decreasing
Wavelength	340 nm
Flow cell temp	37^0C
Zero setting with	Distilled water
Delay time	10 secs
Kinetic interval	60 secs
No.of readings	5
Reagent volume	1 ml
Sample volume	100 µl
Factor	2225
Linearity	500
Units	IU/L
Low normal	0.00
High normal	48.0

ESTIMATION OF ALKALINE PHOSPHATASE

AMP Buffer/IFCC Method

PRINCIPLE

p-Nitrophenyl phosphate analogue + $H_2O \xrightarrow{ALP}$ p-Nitrophenol + Phosphate

ACTIVE INGREDIENTS OF WORKING AGENT

AMP	pH 9.7 3.5 mol/l
Magnesium chloride	0.6 mmol/l
p-Nitrophenyl phosphate analogue	0.070 mmol/l
Sodium azide	0.10%
Zinc sulphate	0.3 mmol/l

Specimen

Specimen is Unhemolysed serum .Samples are stable for a week at 2 - 8^0 C and for a month at -10^0C.The ALP activity in serum stored at 2 - 8^0 C increases with time.

Test Procedure

R1	0.8 ml
R2	0.2 ml
Serum/Plasma	25 µl

Mix well and aspirate. Measure the change in optical density against distilled water at 405 nm as follows

A_0 - Exactly after 60 seconds

A_1 - Exactly after every 60 seconds

Activity of ALP activity in IU/ $= \Delta$ A/min x 320

System Parameters

Reaction type (Mode)	Kinetic
Reaction Direction	Increasing
Wavelength	405 nm
Flow cell temp	37^{0}C
Zero setting with	Distilled water
Delay time	60 sec
Kinetic interval	60 secs
No.of readings	5
Reagent volume	R1 0.8 ml + R2 0.2 ml
Sample volume	25 µl
Factor	3200
Linearity	1000
Units	IU/L

Reference range

20- 50 years Males: 53 – 165 U/L Females : 42 – 136 U/L

≥ 60 years Males : 56 – 136 U/L Females : 53 – 160 U/L

In children the value will be higher than adults i.e 350 – 585 IU/L

RESULTS AND STATISTICAL ANALYSIS

The study population consisting of 100 subjects – 50 controls and 50 cases. Statistical software SPSS was used to analyze the statistical data. The distribution of age among Group I & II were shown in table 1. Estimation of mean and standard deviation done for each group. Data expressed as mean +/- standard deviation.

Students unpaired 't' test is used to compare the means between two independent groups. F test is applied between the study variables to know whether 't' test can be applied to study the parameters and also which type of 't' test –either equal variance or separate variance unpaired 't' test can be applied in this study. Pearson coefficient of correlation is used to estimate the degree of association between two quantitative variables. A p-value of <0.001 will be considered as statistically significant.

Age group wise comparison of mean values of zinc and alkaline phosphatase were done. Graphical representation was done using scatter diagrams.

Table 1

Age distribution among cases and controls

Group	N	Mean age(years)	Standard deviation	Student 't'test
Case	50	42.79	9.68	p value > 0.05 not significant
Control	50	44.8	8.64	

Table 2

Age distribution among cases and controls

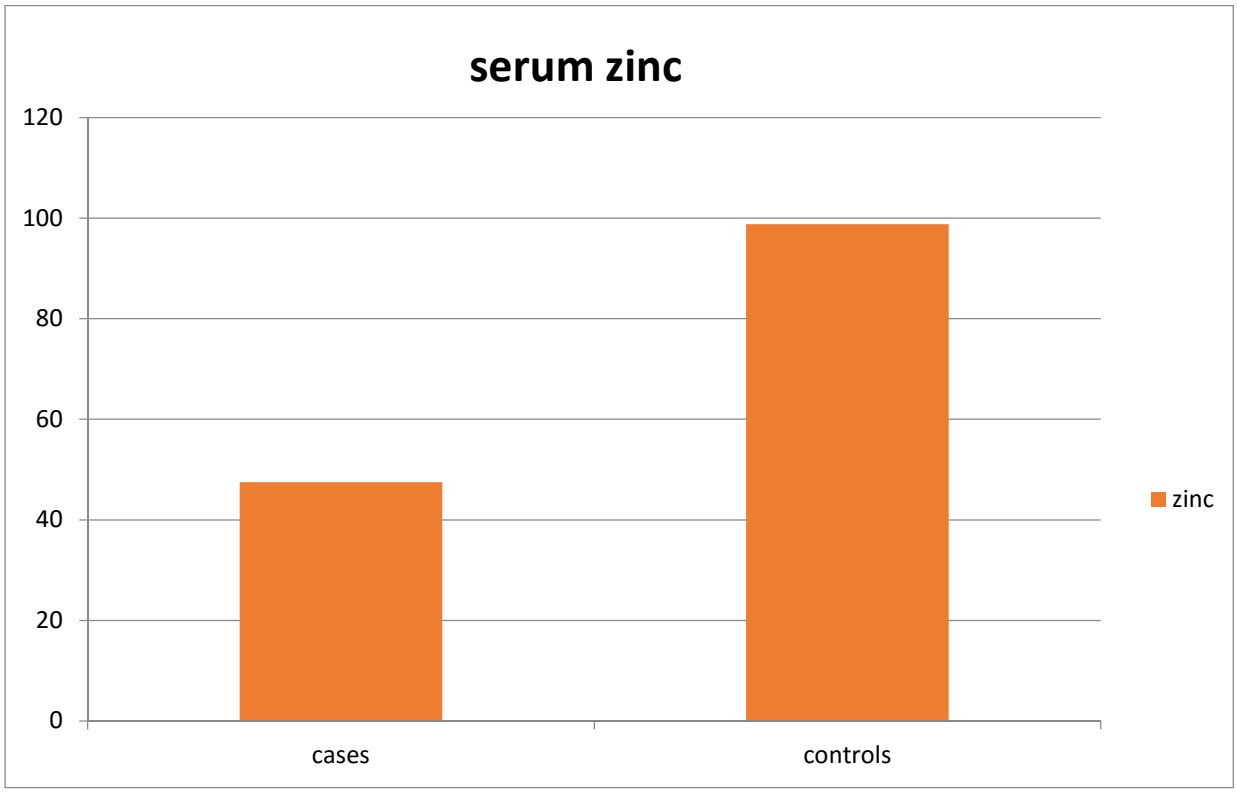
	Case	Control
20-35	11	10
36-50	25	26
51-75	14	14
Total	50	50

Table 3

Mean and standard deviation of Variables in groups

Variable	Mean ± S.D		P value
	Cases	Control	
T.bilirubin	16 ± 7.4	0.5 ± 0.2	< 0.001 significant
Albumin	2.3 ± 0.8	4.8 ± 6.2	
Globulin	3.2 ± 0.8	2.5 ± 0.4	
AST	160 ± 55.4	18.6 ± 11.1	
ALT	71 ± 62	10.8 ± 5.8	
GGT	130.2 ± 112	9.6 ± 5.4	
Alk.phos	234 ± 191.6	89.2±28.4	
Creatinine	1.2±1.02	0.7±0.1	
INR	1.4±0.4	0.9±0.06	
Prothrombin time	17.5± 3.9	11.2±0.6	
Zinc	47.5±19.7	98.8±26	

Comparison of zinc values between cases and controls



Serum zinc in µg /dl

PEARSON'S CORRELATION

The sign of 'r' denotes the nature of association

+ sign – Positive correlation

- sign – Negative correlation

The value of 'r' denotes the strength of association

'r' lies between +1 and -1

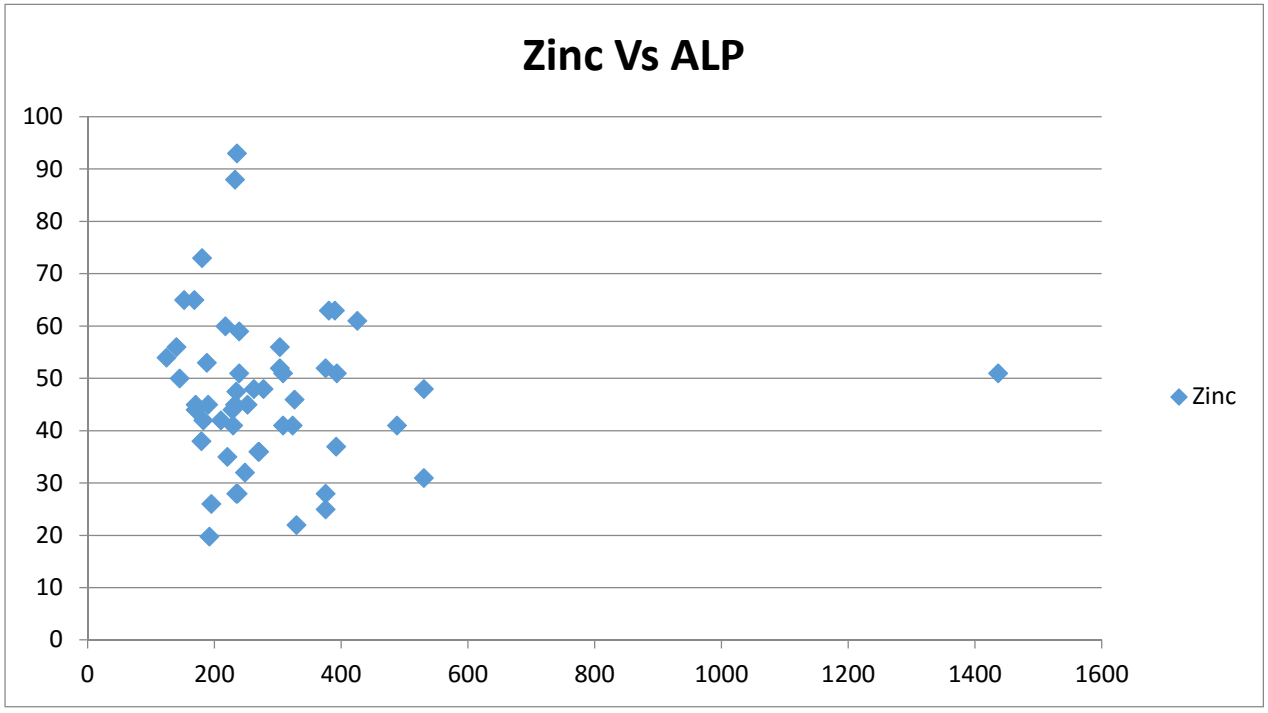
P value < 0.05 indicates significant association

Table 4: Correlation of serum zinc and alkaline phosphatase in alcoholic liver disease patients

Variables	Pearson's correlation coefficient (r)	Significance (p)	Interpretation
Zinc vs Alk.phos	-0.06	<0.001	Significant & negative correlation

Scatter diagram

Correlation of zinc and alkaline phosphatase



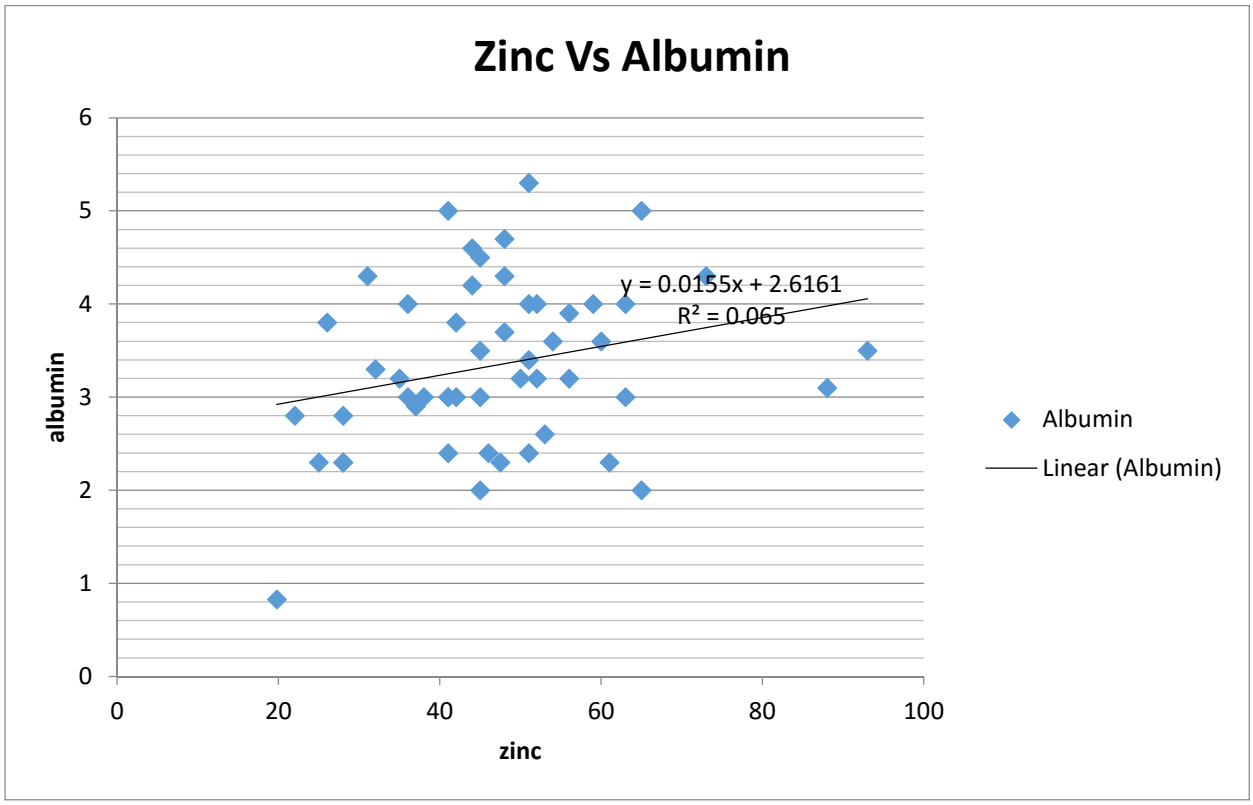
X- axis Zinc µg/dl

Y-axis Alk Phos U/L

Table 5 : Correlation of serum zinc and albumin in alcoholic liver disease patients

Variables	Pearson's correlation coefficient (r)	Significance (p)	Interpretation
Zinc vs Albumin	0.174	<0.001	Significant & positive correlation

Scatter diagram



Zinc in µg/dl ,albumin in g/dl

Table 6 : Correlation of serum zinc and duration of disease in alcoholic liver disease patients

Variables	Pearson's correlation coefficient (r)	Significance (p)	Interpretation
Zinc vs Duration of alcohol intake	-0.603	< 0.001	Significant & negative correlation

Scatter diagram

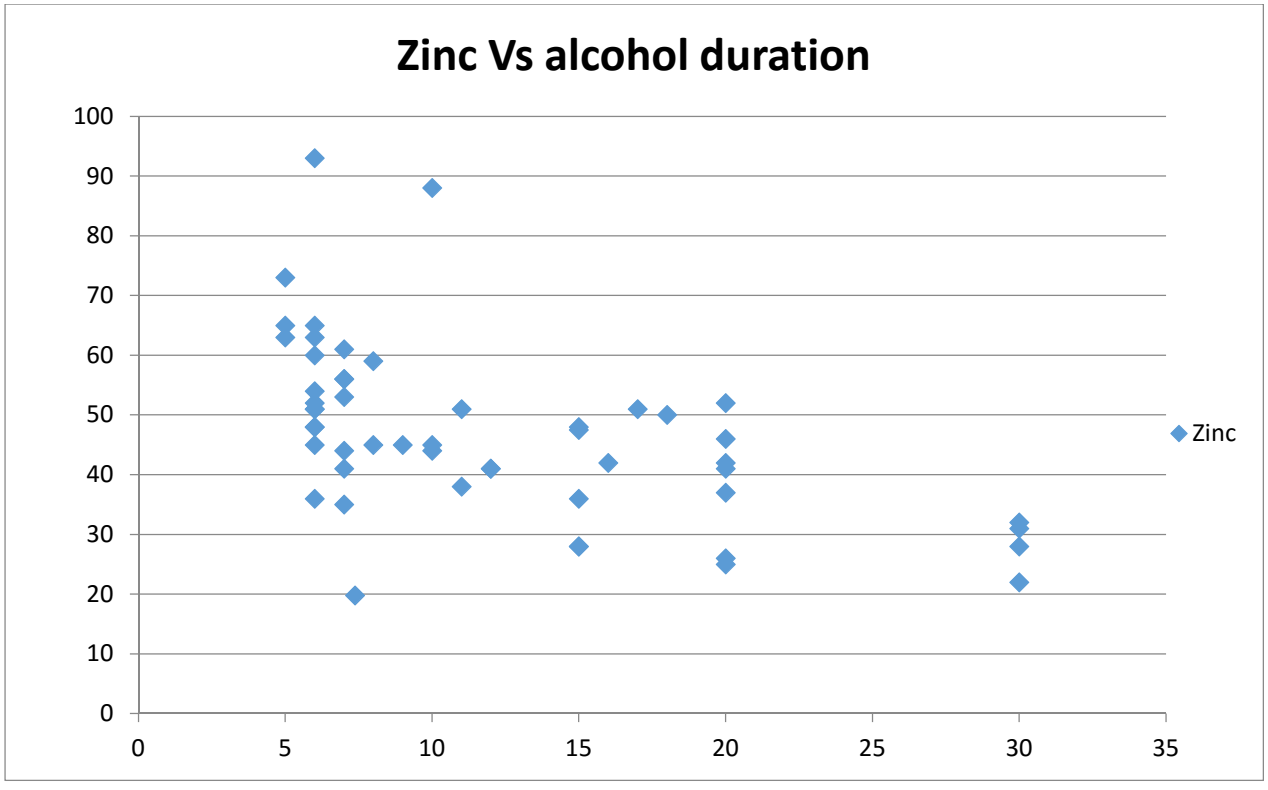


Table 7: Correlation of serum zinc and MELD in alcoholic liver disease patients

Variables	Correlation coefficient (r)	Significance	Interpretation
Zinc vs MELD	-0.185	< 0.001	Significant & negative correlation

Scatter diagram

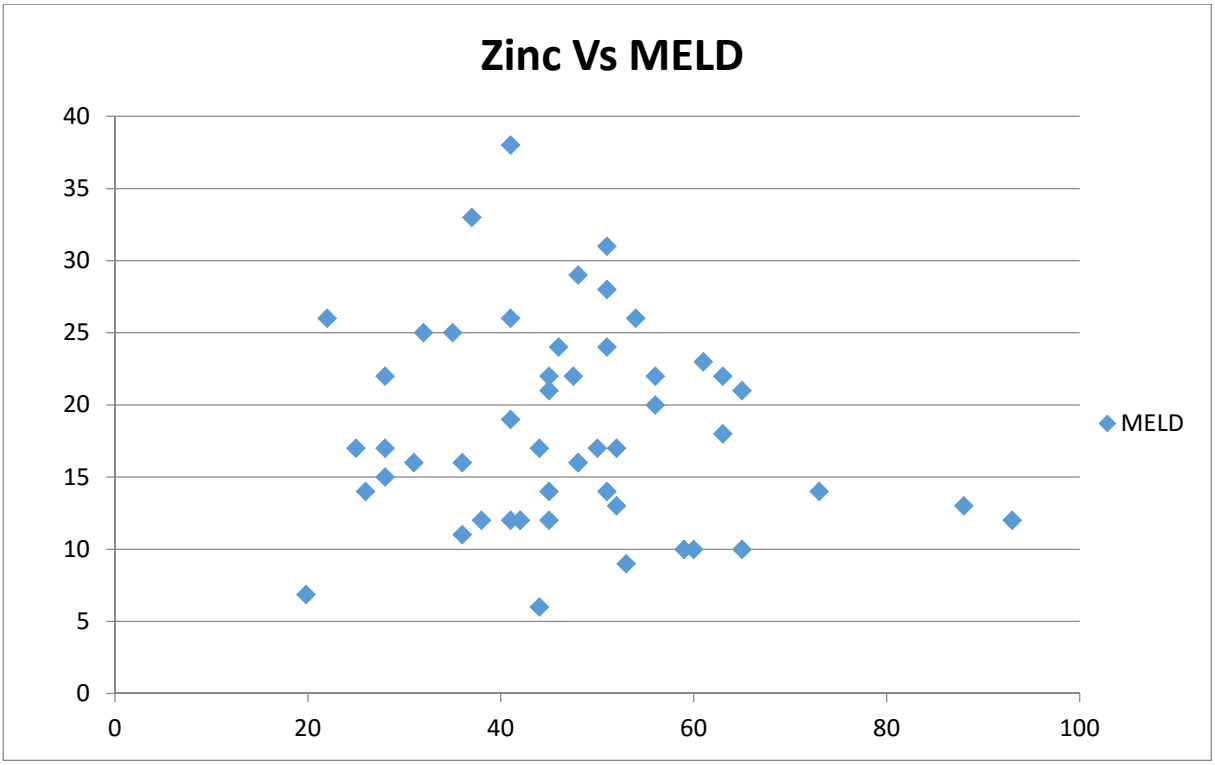


Table 8 : Correlation of serum zinc and GGT in alcoholic liver disease patients

Variables	Correlation coefficient (r)	Significance (p)	Interpretation
Zinc vs GGT	-0.301	<0.001	Significant & negative correlation

Scatter diagram

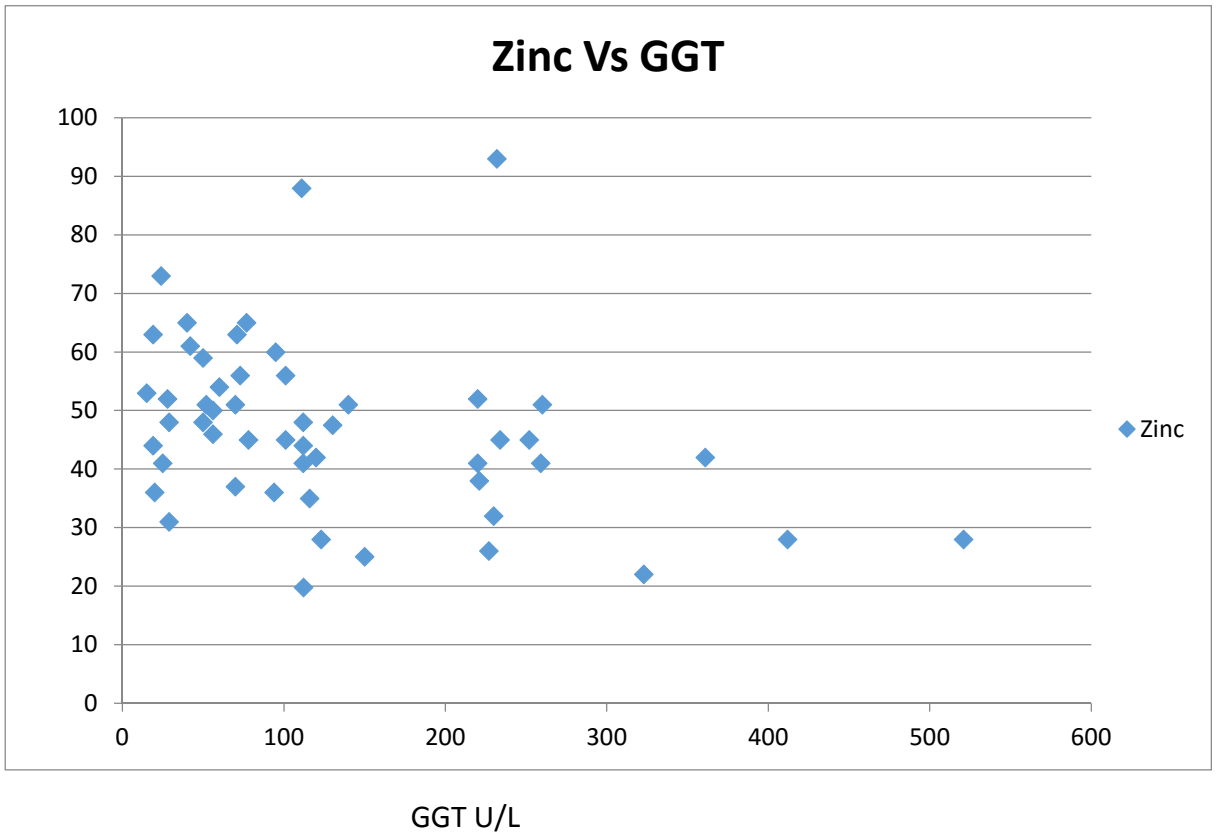
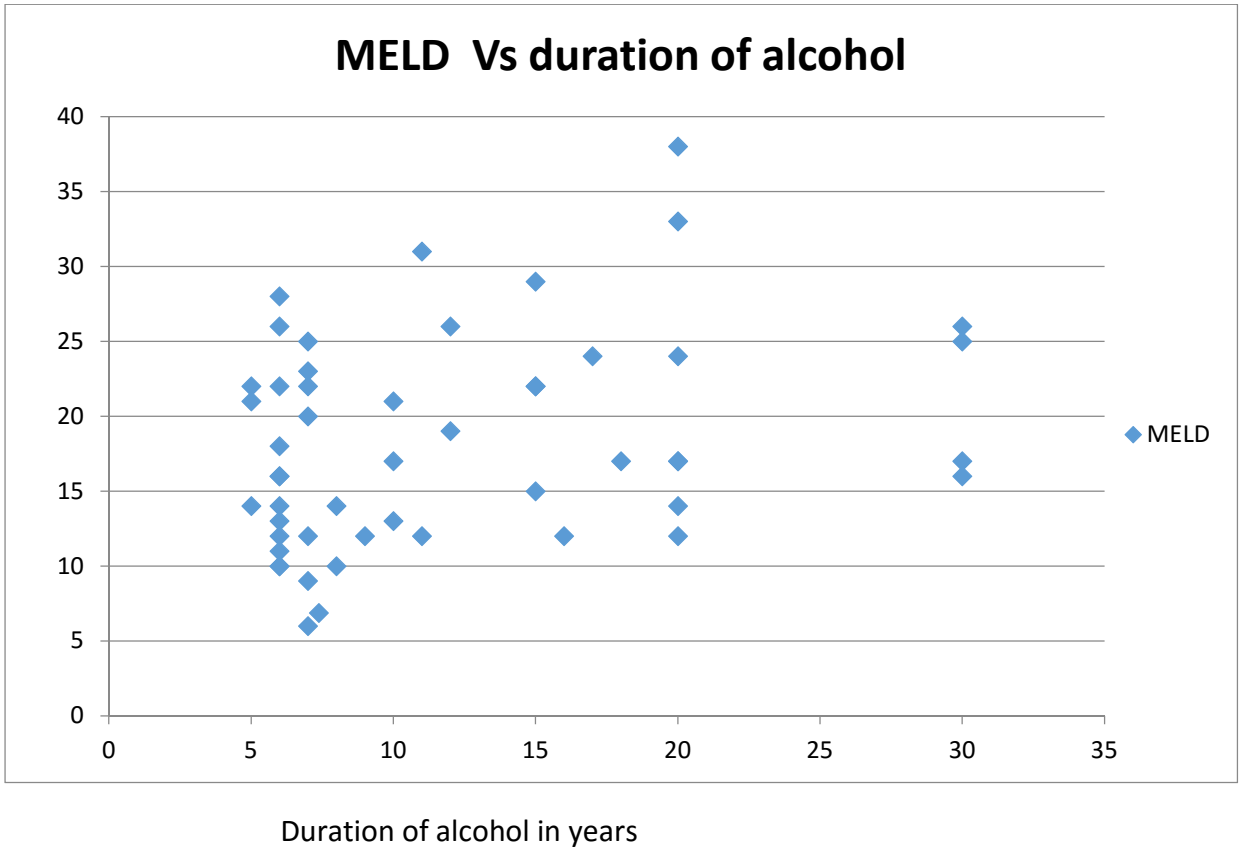


Table 9 : Correlation of MELD and Duration of alcohol in alcoholic liver disease patients

Variables	Correlation coefficient (r)	Significance (p)	Interpretation
MELD Vs duration of alcohol	0.275	<0.001	Significant & positive correlation

Scatter diagram



DISCUSSION

The present study results demonstrate that higher percentage of patients with alcoholic liver disease have low serum zinc levels compared to normal subjects. Ferdousi et al has evaluated the zinc status in patient with liver cirrhosis and has shown significant lower plasma zinc levels[24].

Low levels of zinc in alcoholic liver diseases can be attributed to zinc bound to albumin fraction which is decreased in liver disease. The anorectic effect of alcohol decreases zinc uptake, and also the diuretic effect of alcohol can cause increase loss of zinc in urine[42].

In the present study the comparison between all the parameters of liver function tests show uniform significance between cases and controls.

Azam et al has analyzed various enzyme panel in liver disease and has found increased levels of liver enzymes in various states of liver disease[35].

In our present study there is a significant increase in the levels of GGT and ALP among cases. This increase can be related to chronic alcoholism and obstruction.

In this study there is a decrease in serum albumin levels among cases when compared to controls .This is explained by the derangement of synthetic functions in

chronic liver disease[9]. Few cases show abnormal coagulation parameters like prothrombin time and INR which are increased.

Weismann et al has analysed ALP status in zinc deficient patients of acrodermatititis enteropathica and elderly, as zinc is the cofactor for ALP, and has found ALP levels decreased when zinc levels went down[36].

In the present study there is an increased alkaline phosphatase level in spite of hypozincemia among cases and this can be attributed to the obstruction that occur in the later stages of cirrhotic liver.

The karl pearsons correlation coefficient between serum zinc and alkaline phosphatase showed negative association and trivial correlation.

A significant and negative correlation was found between zinc and duration of the alcohol intake. A significant and negative correlation was seen between zinc and MELD score .This explains that the decrease in the trace element zinc is related to the duration of alcohol intake and progression of the disease.

A significant and positive correlation was found between serum zinc and serum albumin levels. The study by Kaushik kar et al has analyzed the same and had stated a significant p value < 0.001 .This attributes to the bound nature of zinc to albumin[28].

A significant and positive correlation was analyzed between duration of alcohol intake and MELD score. This clearly shows the contribution of alcohol as a hepatotoxic agent to worsen the condition of the patient.

Further study with larger sample size, estimation of urinary levels of zinc, tissue zinc levels, use of gold standard AAS for estimation of zinc are needed to exactly evaluate the status of zinc in these patients and supplementing with zinc to bring down the severity of the disease.

CONCLUSION

In conclusion, the present study reflects the decrease in serum zinc levels and elevated alkaline phosphatase levels in patients with alcoholic liver disease. The significant statistical data provides us a strong rationale for evaluating the zinc status along with the routine liver function profile and also supports the supplementation of zinc to patients with alcoholic liver disease.

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MASTER CHART – CONTROLS

S.No	Age	Sex	Zinc µg/dL	Albumin g/dL	Globulins g/dL	ALP U/L	T.Bilirubin mg/dL	SGOT U/L	SGPT U/L	GGT U/L	PT sec	INR	Creatinine Mg/dL
1	42	M	89	4.2	3	65	0.9	45	21	5	10.5	0.9	0.6
2	36	M	112	3.8	3	63	1	12	5	13	11.9	1.03	0.5
3	41	M	69	3.6	2.8	98	0.5	9	4	4	9.9	0.87	1
4	55	M	110	4.6	3	112	0.6	12	7	7	10.5	0.92	0.6
5	45	M	89	3.9	2	102	0.6	7	4	15	10.6	0.92	0.8
6	32	M	96	4	2	69	0.8	25	12	16	10.4	0.91	0.9
7	42	M	79	4	3	120	1.2	25	7	24	12	1.04	0.9
8	28	M	135	4.3	3	45	0.2	24	22	21	9.9	0.87	0.9
9	52	M	56	3.2	2.8	63	0.3	5	5	12	10.4	0.97	0.9
10	48	M	78	3	3	69	0.5	15	8	3	11.9	0.9	0.7
11	44	M	124	4.1	2.2	86	0.3	5	5	7	11.6	1.02	0.5
12	43	M	100	4.2	2	84	0.3	29	12	14	11.1	0.99	0.9
13	60	M	69	3.5	2.3	76	0.5	22	15	6	10.2	0.94	0.6
14	58	M	78	3.8	2	92	0.2	15	11	5	11.7	0.97	0.5
15	31	M	141	5	2.8	81	0.3	22	7	8	11.4	1.01	1.2
16	59	M	71	3.5	3.1	64	1	24	5	11	10.8	0.91	0.5
17	41	M	103	4.3	3	73	0.6	35	21	16	11.2	0.96	0.6
18	48	M	89	4	2.6	150	1.1	20	21	12	11.6	0.96	0.8
19	49	M	84	4	3	68	0.3	7	9	4	10.4	1.04	0.8
20	43	M	125	4.6	2.6	112	0.6	12	11	12	11	1.03	1
21	52	M	96	3.8	2.8	64	0.6	6	5	6	11.2	0.97	0.9
22	51	M	65	3	3	83	0.4	16	11	12	11.3	0.89	0.9
23	30	M	132	5	2.9	81	0.3	22	7	17	11.4	1.07	0.6
24	38	M	112	48	3	75	0.9	8	5	11	10.8	1.04	0.8
25	57	M	69	3	2.5	98	0.3	40	12	5	10.4	0.98	0.8
26	52	M	70	3.2	2.2	86	0.3	23	16	4	11.6	0.98	0.6
27	45	M	113	4.6	2.2	96	0.8	7	5	5	10.9	1	0.6
28	43	M	120	4.8	2.8	93	0.6	10	5	3	11.2	1.01	0.7
29	48	M	98	3.5	2	150	0.3	23	19	6	11.6	1.02	0.9
30	49	M	104	4	2.5	114	0.5	12	11	4	10.7	0.96	0.5
31	41	M	109	4	2.2	105	0.5	14	14	11	12	0.93	0.5
32	29	M	132	5	2.3	65	1	29	13	24	12.3	1.04	0.5
33	36	M	100	3.6	2	95	0.3	7	5	15	12	0.97	0.8
34	44	M	98	3.9	2.6	102	0.5	5	5	8	11.2	1	0.6
35	58	M	71	3.3	2	52	0.5	44	16	11	10.9	1.04	0.6
36	38	M	140	5.2	2.3	94	1.2	23	11	6	11.4	0.96	0.7
37	45	M	99	4	2.3	65	0.3	12	7	16	11.6	1.07	1
38	46	M	95	4.2	2	85	0.3	10	7	16	11.5	1.09	0.6
39	61	M	56	3	2.8	86	0.6	31	18	11	12	1	0.5
40	58	M	89	3.9	3	74	0.5	22	11	6	11.2	0.9	0.9
41	38	M	112	4	3	110	1	45	21	5	11	1.02	0.5
42	56	M	105	4.2	3.2	125	0.9	22	22	6	11.9	1.04	0.4

43	41	M	116	4.3	2.5	41	0.7	6	5	6	11.1	1.03	0.8
44	32	M	80	3.2	2.8	72	0.3	5	5	12	12.5	1.07	0.8
45	44	M	128	4.5	2	96	0.3	15	11	4	11.1	1	1
46	41	M	122	5	3	69	0.6	32	12	5	12.7	1.1	0.9
47	48	M	107	4	2.1	67	0.6	12	7	10	11.6	1.04	0.8
48	42	M	99	4	2	114	0.3	22	15	2	12.5	1.11	0.8
49	47	M	86	3.8	2.1	200	1.2	8	5	6	11.6	1.04	0.5
50	34	M	123	4.6	3	114	1	29	23	12	11.1	0.9	0.6

MASTER CHART – CASES

No	Age	Sex	Zinc µg/dL	Alb g/dL	Glob g/dL	ALP U/L	Bilirubin mg/dL	SGOT U/L	SGPT U/L	GGT U/L	PT sec	INR	Creat Inine Mg/dL	MELD	Duration of alcohol
1	40	M	36	3	2.5	269	6.6	107	52	94	15	1.2	1	16	15
2	32	M	93	3.5	3	235	2.1	109	47	232	16	1.3	0.9	12	6
3	25	M	73	4.3	2.3	180	3.2	112	28	24	16	1.3	0.9	14	5
4	38	M	41	5	3	229	2.05	149	29	25	16	1.3	0.9	12	7
5	57	M	22	2.8	3.2	329	12.05	149	29	323	20	2	1.3	26	30
6	38	M	45	3.5	3.6	232	2.1	107	47	234	18	1.3	1	12	9
7	52	M	26	3.8	3.4	195	6.6	70	20	227	14	1	0.8	14	20
8	37	M	45	3	3	170	2.1	30	16	78	19	1.6	0.7	14	8
9	53	M	28	2.8	3.2	236	6.9	167	40	412	14	1	1.2	15	15
10	47	M	38	3	3	179	4	132	70	221	11.2	1.02	1	12	11
11	45	M	60	3.6	2.8	217	2.97	106	40	95	14	1	0.5	10	6
12	40	M	56	3.9	4.7	140	5.3	78	26	73	17	1.2	1.8	20	7
13	48	M	42	3.8	2.6	210	3.01	187	40	120	12.7	1.1	1	12	16
14	46	M	63	3	2	380	5.6	145	56	71	19	1.6	0.7	18	6
15	40	M	41	3	3.6	488	17.2	161	60	259	21	1.9	6.9	38	20
16	46	M	51	5.3	1.4	239	3.5	29	28	70	14	1	1.3	14	6
17	30	M	45	4.5	2.5	190	8.8	18	28	101	23	2	0.4	22	6
18	62	M	52	4	3.1	375	4.4	45	25	220	17	1.4	1.1	17	20
19	36	M	35	3.2	2.8	220	7.8	26	19	116	29	2.6	0.4	25	7
20	32	M	88	3.1	3.5	232	2.8	50	37	111	14	1	1.3	13	10
21	45	M	42	3	1.7	182	4.16	82	45	361	11	1	0.8	12	20
22	28	M	36	4	3	270	3.7	78	81	20	14	1	1	11	6
23	36	M	44	4.2	3.5	170	3.1	99	76	112	20	1.8	0.7	17	10
24	32	M	65	2	3.9	152	3.9	121	44	77	22	2.02	1.2	21	5
25	37	M	56	3.2	2.8	303	2.2	25	28	101	17	1.4	2.5	22	7
26	40	M	51	4	4	393	3.1	117	198	52	19	1.8	3	28	6
27	55	M	45	2	3.2	252	3.9	121	65	252	22	2.02	1.2	21	10
28	47	M	54	3.6	1.8	124	22.3	113	235	60	15	1.1	2.1	26	6
29	36	M	41	2.4	5.2	308	28	149	189	112	19	1.6	1.2	26	12
30	35	M	48	4.7	3.3	262	2.4	70	160	50	20	1.8	0.7	16	6
31	40	M	41	3	5	323	3.6	121	41	220	22	2	1	19	12
32	42	M	63	4	4.7	390	4.4	70	54	19	25	2.2	1.1	22	5
33	27	M	61	2.3	3.9	425	15.2	99	129	42	20	1.7	1	23	7
34	47	M	59	4	2.8	239	2.3	76	76	50	11	1	0.8	10	8
35	60	M	25	2.3	4.6	375	4	161	82	150	19	1.6	0.6	17	20
36	54	M	32	3.3	2.5	248	12.2	262	38	230	22	2.2	0.3	25	30
37	51	M	44	4.6	3.2	228	0.49	101	21	19	11	1	0.8	6	7
38	35	M	51	2.4	4.2	308	28	171	189	260	19	1.6	0.7	24	17
39	47	M	48	4.3	3.7	530	2.1	77	221	29	21	1.8	0.9	16	6
40	60	M	28	2.3	4.6	375	4	232	53	521	19	1.6	0.6	17	30
41	47	M	51	3.4	2.7	1436	16.3	130	38	140	21	1.8	2.1	31	11

42	37	M	48	3.7	3.9	277	7.5	117	23	112	22	2	2.16	29	15
43	42	M	52	3.2	2.8	303	2.3	76	25	28	17	1.4	0.8	13	6
44	52	M	37	2.9	2.3	392	22.2	121	198	70	17	1.4	3.2	33	20
45	52	M	31	4.3	4.6	530	2.1	232	221	29	21	1.8	0.9	16	30
46	62	M	53	2.6	1.9	188	0.9	25	49	15	16	1.3	1.2	9	7
47	56	M	50	3.2	2.8	145	1.8	60	36	56	12	1	0.5	17	18
48	35	M	46	2.4	4.2	326	28	165	121	56	16	1.3	1.2	24	20
49	35	M	65	5	3	168	2.3	65	36	40	12	1	0.6	10	6
50	57	M	28	2.3	3.2	234	16	160	45	123	17	1.4	1.2	22	15

PROFORMA

Case No:

Name of the patient: **Age/Sex**

Address:

History Of present illness:

Past History:

Diabetes mellitus: Hypertension: Ischemic Heart disease:
Lung disease Thyroid disease: Dialysis
Liver disease Any other illness

Drug History:

Personal History:

Diet: Smoking Alcohol :

Type :

Amount :

Duration :

FAMILY HISTORY:

PHYSICAL EXAMINATION:

Height weight Pallor Icterus
Clubbing: Cyanosis Lymphadenopathy Thyroid / Parotid/ gynaecomastia /testis/skin Edema

VITALS

BP Pulse Respiratory rate Temp

SYSTEMIC EXAMINATION:

Respiratory System

Cardiovascular System

Abdominal System

Nervous System

INVESTIGATION:

Plasma Zinc:

Serum Alkaline phosphatase:

OTHERS:

Serum T.Bilirubin

D.Bilirubin

SGOT

SGPT

Total Protein

Albumin

A/G RATIO

Prothrombin time

Serum creatinine

தகவல் படிவம்

குடி நோயால் கல்லீரல் பாதிக்கப்பட்ட நோயாளியின் இரத்தத்தில் உள்ள பிளாஸ்மா ஜின்க் ஆளவு மற்றும் ஸீரம் ஆல்கலின் பாஸ்படேஸ் செயல்பாடு ஆகியவற்றை கண்டறியும் ஆய்வு

ஆராய்ச்சி நிலையம்: அரசு ஸ்டான்லி மருத்துவமனை, சென்னை.

ஆய்வு மேற்கொள்பவரது பெயர்: மரு.த.உமா.

மேற்குறிப்பிட்டுள்ள ஆய்வு குடி நோயால் கல்லீரல் பாதிக்கப்பட்ட நோயாகளின் நலனுக்காக அரசு ஸ்டான்லி மருத்துவமனை, சென்னை, உயிர் வேதியியல் துறையை சார்ந்த மருத்துவரால் மேற்கொள்ளப்படுகிறது.

குடி நோயால் கல்லீரல் பாதிக்கப்பட்ட நோயாளியின் (Alcoholic liver disease) இரத்தத்தில் உள்ள பிளாஸ்மா ஜின்க் ஆளவு குறைந்து காணப்படுகின்றது இத்துடன் அவர்கள் உடலில் ஸீரம் ஆல்கலின் பாஸ்படேஸ் நொதியின் செயல்பாடு குறைந்து காணப்படுவதால் இவ்வாராய்ச்சியின் மூலம் இவற்றுக்கிடையேயான தொடர்பை வெளிக் கொணர வேண்டியுள்ளது. மேலும் குடி நோயால் கல்லீரல் பாதிக்கப்பட்ட நோயாளியின் உடலில் உள்ள ஜின்க் குறைபாட்டை ஈடுசெய்யவும் இவ்வாராய்ச்சி உதவும்.

இந்த ஆரய்ச்சி சுய விருப்பத்துடன் பங்கேற்கும் நோயாளிகளுக்கு மட்டும் சோதனை செய்யப்படும். ஆய்வில் பங்கேற்கும் நோயாளிகளின் வழக்கமான மருத்துவ சிகிச்சையை பாதிக்காமல் இந்த ஆய்வு செய்யப்படும்.

ஒப்புதல் படிவம்

ஆய்வின் தலைப்பு

"குடி நோயால் கல்லீரல் பாதிக்கப்பட்ட நோயாளியின் இரத்தத்தில் உள்ள பிளாஸ்மா ஜின்க் ஆளவு மற்றும் ஸீரம் ஆல்கலின் பாஸ்படேஸ் செயல்பாடு ஆகியவற்றை கண்டறியும் ஆய்வு".

ஆராய்ச்சி நிலையம்: அரசு ஸ்டான்லி மருத்துவமனை, சென்னை.

மேற்குறிப்பிட்டுள்ள ஆய்வு விவரங்கள் எனக்கு விளக்கப்பட்டது. என்னுடைய சந்தேகங்களைக் கேட்கவும் அதற்கான தகுந்த விளக்கங்களைப் பெறவும் வாய்ப்பளிக்கப்பட்டது.

நான் இந்த ஆய்வில் தன்னிச்சையாகத் தான் பங்கேற்கிறேன். எந்த காரணத்திலும் எந்த கட்டத்தலும் எந்த சட்ட சிக்கலுக்கும் உட்படாமல் நான் இந்த ஆய்வில் இருந்து விலகிக் கொள்ளலாம் என்றும் அறிந்து கொண்டேன்.இந்த ஆய்வு சம்மந்தமாகவும் இதை சார்ந்த மேல் ஆய்வு மேற்கொள்ளும் போதும் இந்த ஆய்வில் பங்கு பெறும் மருத்துவ/ சமூக பணியாளர் என்னுடைய மருத்துவ அறிக்கைகளைப் பார்ப்பதற்கு என் அனுமதி தேவையில்லை என்றும் அறிந்து கொண்டேன். நான் ஆய்வில் இருந்து விலகிக் கொண்டாலும் இது பொருந்தும் என அறிகிறேன்.

இந்த ஆய்வின் மூலம் கிடைக்கும் தகவல்களையும் பரிசோதனை முடிவுகளையும் மற்றும் சிகிச்சை தொடர்பான தகவல்களையும் மருத்துவ / சமூக பணியாளர் மேற்கொள்ளும் ஆய்வில் பயன்படுத்திக் கொள்ள என் முழு மனதுடன் சம்மதிக்கிறேன்.

இடம்: பங்கேற்பவர்/உறவினர் கையெப்பம்

தேதி: கைவிரல் ரேகை

பங்கேற்பவரின் பெயர் மற்றும் விலாசம் :

ஆய்வாளரின் கையெப்பம் :

ஆய்வாளரின் பெயர் : மரு.த.உமா.

INSTITUTIONAL ETHICAL COMMITTEE,
STANLEY MEDICAL COLLEGE, CHENNAI-l

Title of the Work	: Evaluation of Serum Zinc status and Serum alkaline Phosphatase activity in Alcoholic liver diseases.
Principal Investigator	: Dr. T Uma
Designation	PG in MD (Bio-Chemistry)
Department	Department of Bio-Chemistry Government Stanley Medical College, Chennai-01

The request for an approval from the Institutional Ethical Committee (IEe) was considered on the IEC meeting held on 26.11.2014 at the Council Hall, Stanley Medical College,Chennai-l at 2PM

The members of the Committee, the secretary and the Chairman are pleased to approve the proposed work mentioned above, submitted by the principal investigator.

The Principal investigator and their team are directed to adhere to the guidelines given below:

1. You should inform the IEC in case of changes in study procedure, site investigator investigation or guide or any other changes.
2. You should not deviate from the area of the work for which you applied for ethical clearance.
3. You should inform the IEC immediately, in case of any adverse events or serious adverse reaction.
4. You should abide to the rules and regulation of the institution(s).
5. You should complete the work within the specified period and if any extension of time is required, you should apply for permission again and do the work.
6. You should submit the summary of the work to the ethical committee on completion of the work.

K Vasanthi

MEMBERSECRETARY, IEC
SMC CHENNAI